ABSTRACT

Title of Document: THE ROLE OF ORGANIC MATTER IN THE DISSOLVED PHASE SPECIATION AND SOLID PHASE PARTITIONING OF MERCURY

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The interaction of mercury (Hg) and methylmercury (MeHg) with organic matter is extremely important in the dissolved phase speciation and solid phase partitioning of Hg and MeHg in aquatic systems. This study shows, that under oxic conditions Hg and MeHg will likely associated with Fe oxides through an indirect association with organic matter, while under sulfidic conditions, solid phase Fe sulfide will dominate the complexation of Hg to the solid phase. As a result of the association of Hg with Fe solids, which undergo dynamic changes at redox interfaces in aquatic systems, the distribution of Hg on particles is likely changing at redox boundries, areas that have been shown as active zones of methylation. Redox zones are also going to be important in controlling the mobility of MeHg from the site of production to areas in aquatic systems in which uptake by biota occurs.

Although the dissolved phase speciation of Hg has been shown as an important factor in Hg methylation, as a result of the diffusive uptake of neutral Hg-sulfide into

bacterial cells, this speciation had previously not been measured. Hg forms stronger bonds with reduced sulfide relative to dissolved organic matter (DOM), therefore, it was not previously thought that DOM was important in the speciation of Hg under sulfidic conditions. Using modified octanol-water partitioning extractions and centrifugal ultrafiltration, the speciation of Hg in sulfidic natural samples and laboratory solutions was examined. It was shown that the concentration of neutral Hg-sulfide complexes are lower than predicted by thermodynamic models, as a result of an interaction of these species with DOM. It is proposed that the interaction of Hg with DOM is not a complexation, but rather, a partitioning of neutral Hg-sulfide complexes into hydrophobic portion of the DOM. Thermodynamic constants were calculated for this interaction and applied to model the speciation of Hg in natural samples. The concentration of neutral Hg-sulfide is lower than models previously predicted, as a result of the DOM interaction. Since the concentration of neutral Hg-sulfide affects methylation, DOM could impact the rate of Hg methylation in aquatic systems.

THE ROLE OF ORGANIC MATTER IN THE DISSOLVED PHASE SPECIATION AND SOLID PHASE PARTITIONING OF MERCURY

By

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Dissertation submitted to the Faculty of the Graduate School of the
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Chapter 1: Introduction

1.1. General mercury background

1.1.1. Mercury in the environment

As a result of anthropogenic sources, mercury deposition to aquatic systems has increased over the past century (Mason et al. 1994; Fitzgerald et al. 1998). Mercury is emitted into the atmosphere primarily as inorganic mercury (Hg) and, as a result of its relatively long residence time (~1 year), is deposited in both disturbed and pristine environments (Fitzgerald et al. 1998). While Hg is the dominant form of mercury deposited into aquatic environments, health risks to both humans and wildlife are primarily associated with the consumption of fish containing elevated levels of methylmercury (MeHg) (EPA 1997). MeHg, on average, composes less than 1% of the total concentration of mercury in aquatic sediments (Benoit et al. 2003), yet because of its chemical properties, MeHg bioconcentrates through the food web resulting in MeHg concentrations several orders of magnitude higher in fish relative to the water (Watras and Bloom 1992). Consumption advisories as a result of MeHg levels in fish comprise 75% of all fishery advisories in the United States (EPA 2002). A better understanding of the processes controlling the formation of MeHg across ecosystems is required in order to understand the factors important in the accumulation of MeHg in aquatic organisms.

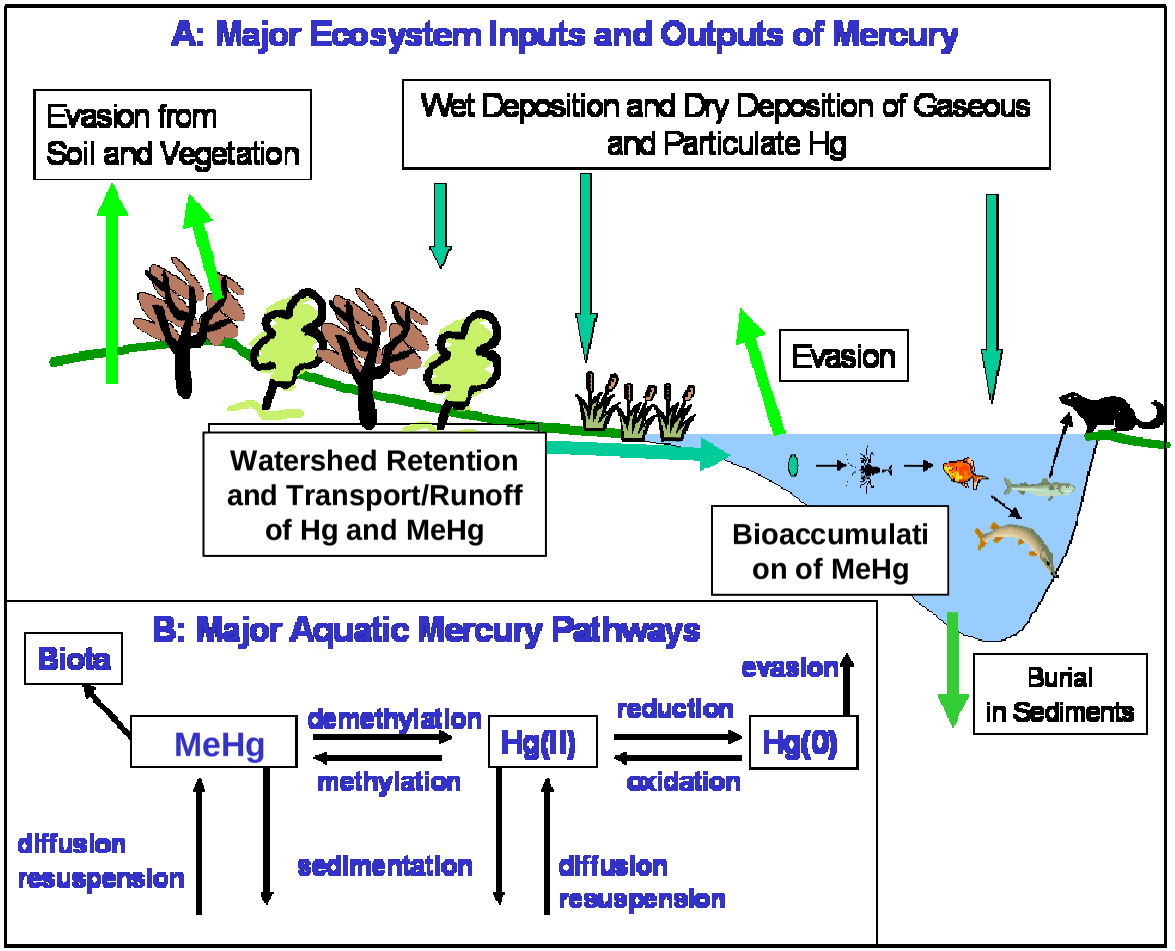


Figure 1.1: Important processes affect Hg and MeHg cycling in aquatic environments. Modified from Mason et al. 2005.

In situ production of MeHg, in both sediments and wetlands, controls the MeHg concentration in the water column and sediment of aquatic systems (Krabbenhoft et al. 1995; St Louis et al. 1996; Branfireun et al. 1998). Figure 1.1 shows the important processes affecting Hg movement through aquatic systems. Hg associated with either the dissolved or particle phase is transported into aquatic systems through atmospheric deposition and terrestrial runoff. Once in the water column, Hg is distributed between the particulate and dissolved phase and can be transported to the sediment, the major Hg reservoir in aquatic systems. In the sediment, Hg partitions between the porewater and the particulate phase. The sediment is thought to be the primary site of Hg methylation in most aquatic systems. MeHg undergoes similar cycling as Hg with the exception that in situ production is the dominant source of MeHg (Morel et al. 1998). Demethylation, the conversion of MeHg to Hg, is also important in the cycling of MeHg in aquatic systems (Marvin-DiPasquale et al. 2000; Benoit et al. 2003).

1.1.2. Mercury speciation controls on methylmercury production

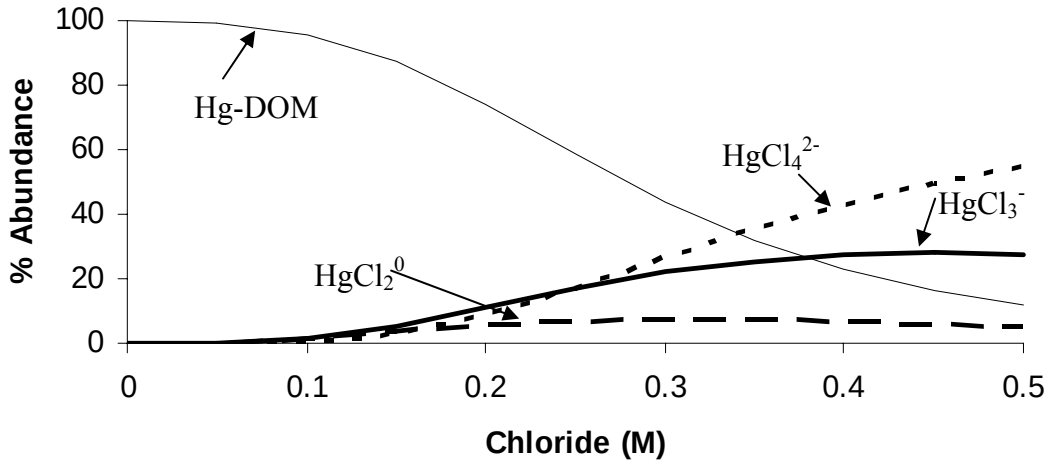
The concentration of MeHg in aquatic organisms is dependent on the net production of MeHg in the aquatic systems, with biotic production of MeHg being substantially more important than abiotic processes (St Louis et al. 1996; Driscoll et al. 1998). Biotic production of MeHg is mediated by sulfate reducing bacteria (SRB) (Compeau and Bartha 1985; Gilmour et al. 1992; King et al. 2001) and since SRB exist in anoxic regions, a majority of Hg methylation occurs in the sediment or anoxic zones of aquatic systems. Large variability in MeHg concentrations are observed

across ecosystems, which cannot be explained solely by differences in Hg concentrations (Benoit et al. 2003). Since MeHg is produced by SRB, bacterial community composition and sulfate reduction rates factor into the methylation rate in a system (Devereux et al. 1996; Macalady et al. 2000; King et al. 2001), but increases in sulfate reduction are not always correlated with increased Hg methylation. The concentration of methylmercury in aquatic systems is also dependent on the rate of MeHg demethylation. Demethylation, which can occur through both biotic and abiotic processes, can also contribute to the variability of MeHg concentrations observed across ecosystems (Marvin-DiPasquale et al. 2000; Benoit et al. 2003).

High sulfide concentrations have been shown to inhibit Hg methylation. (Compeau and Bartha 1985; Benoit et al. 2001c). Benoit and coauthors (Benoit et al. 1999b; Benoit et al. 2001c) showed that the uptake, and therefore the methylation of Hg by SRB, is controlled by the dissolved phase speciation of Hg and that neutral Hg species appear to be preferentially methylated by bacteria. Under oxic conditions, with the exception of high salinity environments, the speciation of Hg is dominated by Hg-DOM complexes (Figure 1.2a) (Benoit et al. 2001a; Ravichandran 2004). As a result of the strong affinity of Hg to sulfide, Hg-sulfide complexes dominate Hg speciation under anoxic conditions, with pH and sulfide concentration controlling the abundance of the different sulfide species (HgS^0, $Hg(SH)_2^0$, $Hg(SH)^+$, HgS_2^{2-}, and $HgHS_2^-$) (Figure 1.2b). Inhibition of Hg methylation is observed at high sulfide concentrations due to the formation of negatively charged Hg-sulfide complexes, which cannot diffuse through the cell membrane of SRB (Benoit et al. 1999b). It should be noted that facilitated uptake of charged Hg species into cells of

Figure 1.2:a) Percent abundance of Hg species under oxic conditions across a chloride gradient assuming a DOM concentration of 10 mg C/L. The stability constant for a DOM isolated from the Florida Everglades (2BS) determined in Benoit et a. 2001 was used in the speciation calculation. B) Percent abundance of different Hg-sulfide complexes across a sulfide gradient. The speciation calculations under both oxic and sulfidic conditions were run at pH 6.0

a)



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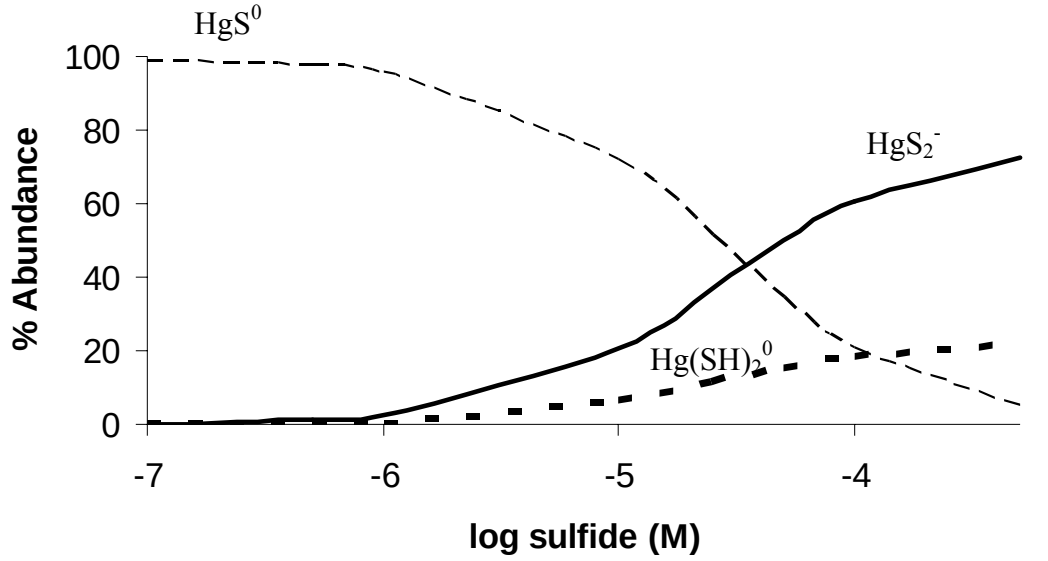


microorganisms has been observed (Golding et al. 2002; Kelly et al. 2003; Najera et al. 2005) but the uptake of charged Hg complexes has never been demonstrated in a microorganism that is known to methylate Hg.

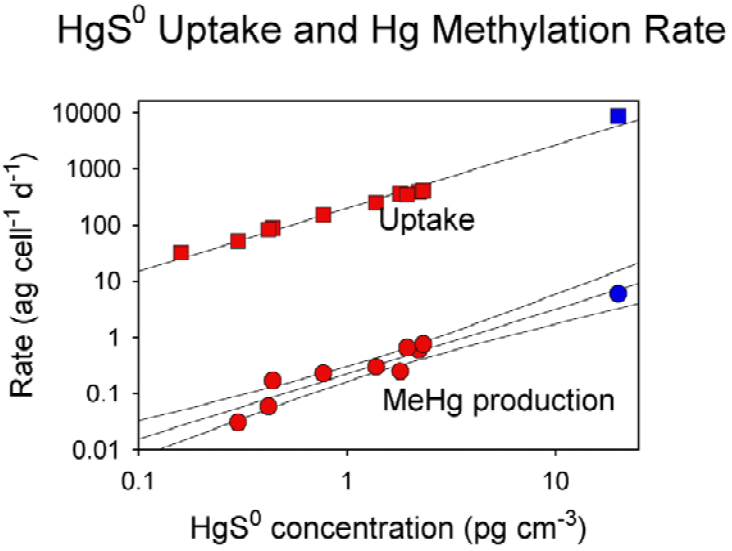
Using octanol-water partitioning coefficients, the cellular permeability of neutral Hg species has been calculated and used to estimate the rate of uptake of Hg into bacterial cells. The calculated diffusion rate of HgS^0 into SRB cells exceeds measured methylation rates by several orders of magnitude (Figure 1.3a). Based on these calculations, it has been concluded that the uptake of Hg by bacterial cells is not the limiting step in the methylation process (Benoit et al. 2001b). Using quantum mechanical calculations, Tossell (2001) determined that HgS^0 is not stable as an aqueous complex and would actually exist as $HOHgSH^0$. As a result of the larger molar volume, calculated diffusion rates for $HOHgSH^0$ are smaller relative to HgS^0, but the calculated diffusion rate is still in excess of the amount of Hg being methylated (Benoit et al. 2003). It has been argued that a potential reason for lower measured methylation rates relative to calculated diffusion rates of Hg into the cell is a result of the sequestration of Hg inside the cell, rendering it unavailable for methylation (Figure 1.3b) (Benoit et al. 2003). However, other explanations are possible, as discussed below.

1.1.3. Mercury speciation under sulfidic conditions

Several studies have examined the complexation of Hg with DOM in order to determine the complexation constants of Hg-DOM complexes and a review of these studies is summarized in Ravichandran (2004). From these studies the complexation

Figure 1.3: a) Estimated diffusive uptake rate of neutral Hg (square points) and measured MeHg production rates (circles) across a gradient of HgS^0 concentrations. b) Processes controlling the uptake and methylation of Hg in a cell. The uptake rate (k_d) is a function of the concentration of the neutral Hg-sulfide species. Once inside the cell, Hg can bind to the cell (k_B) or be converted to MeHg (k_M). Figures modified from Benoit et al. 2003

a)



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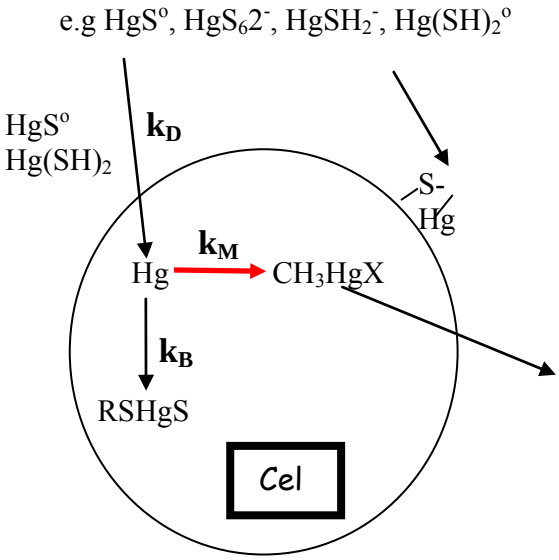


constant of Hg to different isolated and whole DOM fractions were shown to range from 10^{22}-10^{28}. The large range observed in the measured complexation constants has been attributed to variability in the DOM composition and differences in experimental method used to estimate the complexation constants (Ravichandran 2004). It has been demonstrated that reduced sulfur functional groups on the DOM are responsible for the strong complexation of Hg to DOM ((Xia et al. 1999; Benoit et al. 2001a; Haitzer et al. 2002; Ravichandran 2004). Taking a different approach to examine the interaction of Hg with DOM in natural samples, several researchers have used tangential flow ultrafiltration to examine the complexation of Hg to different size classes of DOM in estuarine and freshwater systems. Both the dissolved and colloidal phases were shown to be important in the complexation of Hg with the relative importance of the two phases varying with location and season (Babiarz et al. 2000; Babiarz et al. 2001; Choe et al. 2003). It is difficult to compare the distribution of Hg between the colloidal and dissolved phases across ecosystems because a different molecular weight cutoff was used in the various studies to distinguish between the dissolved and colloidal phases. Babiarz and coworkers used a molecular weight cutoff of 10 kDa, while Choe et al. 2003 used a cutoff of 1 kDa to differentiate between the dissolved and colloidal phase. It is evident from studies examine the complexation of Hg with DOM that Hg-DOM complexes are stronger and therefore more important than inorganic Hg complexes in most natural water systems (Ravichandran 2004).

Even though the speciation of Hg in the presence of sulfide is extremely important in the Hg methylation process, Hg speciation has never been measured in

the environment under natural sulfidic conditions. In all cases where studies have examined the effect of speciation, thermodynamic models have been required to estimate Hg speciation under sulfidic conditions. Thermodynamic models rely on known thermodynamic complexation constants under both oxic and anoxic conditions (Table 1.1). Although Hg binding to DOM is strong, DOM is a weaker ligand relative to sulfide, in the complexation of Hg, and, based on these model predictions, should not affect the speciation of Hg under sulfidic conditions (Benoit et al. 2001a; Ravichandran 2004). However, a recent study indicated that Hg might be interacting with dissolved organic matter under sulfidic conditions (Hsu-Kim and Sedlak 2005). Such an interaction is not currently predicted by the existing thermodynamic models. In that study, C-18 solid phase extractions with a competitive ligand were used and an interaction was observed between Hg, sulfide and DOM (Hsu-Kim and Sedlak 2005). It could not be determined from that study if the observed interaction was actually occurring or if it was an artifact of the experimental design, but the potential interaction of DOM with Hg under sulfidic conditions merits further examination in light of the importance of Hg speciation on Hg methylation. It should also be noted that DOM has been shown to reduce the formation and enhance the dissolution of cinnabar and metacinnabar, $HgS_{(s)}$ (Ravichandran et al. 1998; Ravichandran et al. 1999; Waples et al. 2005), providing farther evidence that DOM can influence the dynamics of Hg in the presence of sulfide. If Hg is interacting with DOM under sulfidic conditions, the concentration of dissolved neutral Hg-sulfide species could be less than previously calculated. As a result, the amount of Hg available for uptake by bacterial cells could, therefore, also be less than thermodynamic models predict,

providing another explanation why measured methylation rates are less than the currently estimated diffusion rate of Hg into cells.

1.1.4 Interaction of mercury with particles

As a result of the high particle affinity of Hg and MeHg, the interaction of Hg with different sedimentary solid phases is important in the cycling of mercury in aquatic systems. Understanding the association of Hg with particles is an essential step in elucidating the processes controlling the methylation of Hg, since currently evidence supports that only dissolved phased Hg is available for Hg methylation (Benoit et al. 2003). Also, the interaction of MeHg with particles will have a significant impact on the transport of MeHg from the site of methylation to zones in the aquatic system where biological uptake of the MeHg occurs. Solid phase distribution coefficients (K_d's) for Hg (log K_d =4.1-5.7 L kg^{-1}) (Bloom et al. 1999a; Mason et al. 1999a; Lawson et al. 2001b; Turner et al. 2004b) are higher than most metals (Lawson et al. 2001b), as a result of the high reactivity of Hg to the particulate phase. Partitioning coefficients for MeHg (log K_d = 2.15-5.32) are usually lower and more variable across ecosystems compared to the K_d's of Hg (Bloom et al. 1999a; Mason et al. 1999a; Lawson et al. 2001b; Kim et al. 2004).

Previous research examining the association of Hg with inorganic solid phases under oxic conditions has primarily focused on the association of Hg with Fe oxides even though Fe oxides only constitute a small fraction (~5% by wt in marine sediments) of the total inorganic phases. Quartz and clay minerals dominate the inorganic solid phases in most environments but these inorganic solids do not

undergo the redox cycling which is observed with Fe and manganese (Mn) oxides (Shchulz and Zabel 2000). The redox cycling of Fe and Mn oxides have been cited as important controllers on the partitioning of Hg in sediment (Bloom et al. 1999a; Mason et al. 1999a; Lawson et al. 2001b; Heyes et al. 2004; Turner et al. 2004b). Both Fe oxide and Mn oxide have large surface areas, 159-234 m^2g^{-1} and 260 m^2g^{-1} respectively, and since the sorption of metals to solid phases is a surface interaction, the importance of Fe and Mn oxides are significant even though they constitute a relatively small fraction of the total inorganic solid phase in sediments. In comparison, clay minerals have been estimated to have a surface area of approximately 30 m^2g^{-1} (Shchulz and Zabel 2000) and quartz has an approximate surface area of 5 m^2g^{-1} (Davis 1982; Tiffreau et al. 1995). Fe oxide is likely more important than Mn oxide in the partitioning of Hg and MeHg in the sediment since Fe is approximately 50 time more abundant relative to Mn in marine sediments (Shchulz and Zabel 2000).

Along with Fe oxide, organic matter has also been cited as an important solid phase in the association of Hg and MeHg with particles (Iverfeldt 1988; Dzombak and Morel 1990; Mason et al. 1993; Quemerais et al. 1998). The interaction of Hg and MeHg with both dissolved and solid phase organic matter is controlled by the interaction of Hg with reduced thiol functional groups on the organic matter (Dyrssen and Wedborg 1991; Xia et al. 1999; Benoit et al. 2001a; Qian et al. 2002; Ravichandran 2004; Zhang et al. 2004). While the complexation constants of Hg to organic matter have been measured in several studies, these measured constants span several orders of magnitude (Table 1.1) as a result of different techniques used to

examine this interaction (Ravichandran 2004). The complexation of MeHg with organic matter has not been as extensively studied, but as is common with the complexation of MeHg with most ligands, the complexation of MeHg with organic matter is weaker than the interaction of Hg with organic matter (Amirbahman et al. 2002; Qian et al. 2002; Karlsson and Skyllberg 2003).

While the interaction of Hg with organic matter has been extensively studied, the relative importance of organic matter and oxides in the complexation of Hg and MeHg has not been addressed. The direct adsorption of Hg with Fe oxide has been examined and complexation constants for this interaction have been determined (Dzombak and Morel 1990; Gunneriusson et al. 1995; Tiffreau et al. 1995). It has been suggested that the interaction of Hg with particles in the environment is a result of a ternary complex between Hg, DOM and Fe oxides but this has never been directly demonstrated (Regnell et al. 1997). Ternary complexes have been shown to exist, using extended X-ray absorption fine structure (EXAFS) spectroscopy, between Fe oxide, Hg and inorganic ligands such as chloride and sulfate (Kim et al. 2004). Under natural conditions, it is extremely likely that Fe oxide particles are coated with organic matter (Mayer 1999) which would result in the interaction of Hg with Fe oxide particles being very different that the direct interaction of Hg with Fe oxides.

It has been suggested that under anoxic conditions, Fe sulfides are the dominant solid phases contributing to the partitioning of Hg to solids (Huerta-Diaz and Morse 1992). In a simple model developed to examine the partitioning of Hg between the solid and dissolved phase in the sediment (Benoit et al. 1999b), it was determined that in order to fit the model to field data, two types of binding sites were

needed for Hg under sulfidic conditions. While the binding sites were modeled as reduced sulfur surface sites on solids, organic and inorganic reduced sulfur sites were not differentiated. Pyrite and acid volatile sulfides (AVS), which include amorphous Fe sulfides, are the dominant Fe sulfides in the sediment (Morse et al. 1987a). Hg has been shown to exhibit a high degree of trace metal pyritization, a measure of the relative association of a metal with pyrite and the AVS solids, which could be a result of coprecipitation or surface adsorption of Hg with pyrite (Huerta-Diaz and Morse 1992). AVS phases are more reactive than pyrite in the sediment (Morse 1991; Zhuang et al. 1994) and consequently, the association of Hg with AVS phases could have a large influence on the distribution of Hg between the porewater and solids. While field observations have been made suggesting the importance of Hg sulfides in the partitioning of Hg under anoxic conditions, direct measurements of this interaction have not been preformed. Also, the influence of organic matter on the partitioning of Hg under sulfidic conditions has not been investigated.

1.1.5 Importance of iron, sulfur and organic matter on the production and fate of methylmercury

While iron, sulfur and organic matter individually have been shown to have significant impacts on the cycling of Hg throughout aquatic systems, the interplay between the three has not been closely studied. Iron, sulfur and organic matter influence both the particle association and dissolved phase speciation of Hg, two processes that are important in the methylation of Hg. The deposition of Hg into aquatic systems is primarily to the surface waters, but as a result of the particle affinity of Hg, it is delivered to the sediment, the primary site of Hg methylation.

Under oxic conditions, organic matter and oxides are thought to be the major solid phases responsible for the association of Hg with particles (Bloom et al. 1999a; Mason et al. 1999a; Lawson et al. 2001b; Heyes et al. 2004; Turner et al. 2004b), while DOM controls the dissolved phase speciation (Ravichandran 2004). Currently, the relative contribution of oxides and organic matter in the partitioning of Hg under oxic conditions is not known.

At redox boundaries in aquatic systems, dynamic changes occur in both the dissolved phase speciation of Hg and the composition of particles, both of which might contribute to the production of MeHg in these zones. Since it is thought that only Hg in the dissolved phase is available for methylation, the distribution of Hg between the particles and the porewater could influence Hg methylation (Benoit et al. 2003). At redox interfaces Fe oxides are reduced, resulting in the release of Fe and anything associated with these particles into the dissolved phase. As sulfide concentration in the sediment increase, as a result of sulfate reduction, Fe sulfide particles form (Morse et al. 1987a). Given the high affinity of Hg to reduced sulfide, these particles have the potential to strongly influence the solid/dissolved phase distribution of Hg (Benoit et al. 2001b), but the relative affinity of Hg for these particles and particulate organic matter has not been examined.

The dissolved phase speciation of Hg has been shown as an important factor in the bacterial production of MeHg, with DOM and sulfide being the two most important ligands in most systems. Thermodynamic models suggest that DOM is not important in the speciation of Hg in the presence of sulfide as a result of the stronger interaction of Hg with sulfide relative to DOM (Benoit et al. 2001a; Ravichandran

2004). Recent work however suggests that DOM might be influencing the speciation of Hg in the presence of sulfide (Hsu-Kim and Sedlak 2005) but this interaction has not been directly examined and could change our understanding of the factors controlling the production of MeHg.

Once produced, the fate of MeHg in aquatic systems will depend on its distribution between the solid and dissolved phase. As mentioned, the production of MeHg occurs in sulfidic regions of aquatic systems, but the uptake of MeHg by aquatic organisms is most likely to occur in oxic regions of the system. As with Hg, it is likely that iron, sulfur and organic matter will influence the partitioning of MeHg between the dissolved and particulate phase. To truly understand the partitioning of Hg and MeHg in the sediment, and the dissolved phase speciation of Hg, the synergistic affect of iron, sulfur and organic matter needs to be examined.

1.2. Research Hypothesis

Based on this review of the literature, and given the present understanding of Hg and MeHg biogeochemistry, the following hypotheses were developed.

1) In the presence of solid Fe sulfide phases, Hg and MeHg will strongly sorb to the solid phase because Hg and MeHg form strong complexes with reduced sulfide ligands
2) The presence of dissolved organic matter in natural systems will reduce the sorption of Hg and MeHg to iron oxides in a concentration-dependant manner, because Hg and MeHg complexation with DOM is stronger than with iron oxides

3) The presence of dissolved organic matter in natural systems will not affect the sorption of Hg and MeHg to solid iron sulfides, because Hg and MeHg complexation with DOM is much weaker that with iron sulfide
4) The presence of dissolved organic matter in natural systems will not affect the speciation of dissolved mercury under sulfidic conditions, because the complexation of Hg with DOM is much weaker than with dissolved sulfides

1.3. Research Objectives

Based on the above hypotheses, the following research objectives were defined, and the research detailed in this dissertation was designed to answer these questions specifically.

1) Determine the relative importance of Fe solid phases and organic matter in the solid/dissolved phase distribution of Hg and MeHg
2) Measure and understand the dissolved phase speciation of Hg in natural sulfidic water samples

1.3.1 Research Approach

In order to address the first research objective, the solid phase partitioning of Hg with Fe oxide and Fe sulfide was examined using synthetic hydrous ferric oxide (HFO) and amorphous Fe sulfide in the presence and absence of DOM. From these experiments, complexation constants were determined to explain the interaction of Hg and MeHg binding with HFO and amorphous $FeS_{(s)}$. Using the laboratory

determined stability constants, along with previously determined constants for Hg and MeHg to organic matter, the relative importance of different solid phases for the partitioning of Hg and MeHg in the sediment was examined (Chapter 2).

The dissolved phase speciation of Hg was measured in natural samples using octanol-water partitioning, in order to address the second research objective. To accomplish this, modifications were required to the previously used octanol-water extraction techniques so that this method could be applied to low level Hg environmental samples. These modifications included the addition of an extra solvent extraction step to remove residual octanol from the water samples in order to avoid analytical interferences of the octanol on the Hg determination (Chapter 3).

Using the modified octanol-water partitioning method, along with centrifuge ultrafiltration, the speciation of Hg in natural water and laboratory prepared solutions was examined in order to measure the Hg speciation under sulfidic conditions. The measured speciation was then compared to thermodynamic speciation models, based on the complexation constants in Table 1.1. It was determined that the speciation of Hg under sulfidic conditions did not agree with thermodynamic model predictions as a result of the influence of DOM with Hg-sulfide complexes (Chapter 4). In order to understand the mechanism controlling the interaction of DOM with Hg-sulfide complexes and to determine stability constants for these species, laboratory studies were undertaken using octanol-water partitioning and ultrafiltration. The conditional complexation constant for the Hg-sulfide-DOM interaction was than used to model the dissolved phase speciation of Hg in different aquatic systems (Chapter 5).

Table 1.1: Hg and MeHg complexation constants

Reaction	***Log K***	Reference
$Hg^{2+} + OH^- = HgOH^+$	10.6	Stumm and Morgan 1996
$Hg^{2+} + 2OH^- = Hg(OH)_2$	21.8	Stumm and Morgan 1996
$Hg^{2+} + 3OH^- = Hg(OH)_3^-$	20.9	Stumm and Morgan 1996
$Hg^{2+} + Cl^- = HgCl^+$	7.2	Stumm and Morgan 1996
$Hg^{2+} + 2Cl^- = HgCl_2$	14.0	Stumm and Morgan 1996
$Hg^{2+} + 3Cl^- = HgCl_3^-$	15.1	Stumm and Morgan 1996
$Hg^{2+} + 4Cl^- = HgCl_4^{2-}$	15.4	Stumm and Morgan 1996
$Hg^{2+} + Cl^- + OH^- = HgOHCl$	18.1	Stumm and Morgan 1996
$Hg^{2+} + CO_3^{2-} = HgCO_3$	12.1	MINEQL
$Hg^{2+} + 2CO_3^{2-} = Hg(CO_3)_2^{2-}$	15.6	MINEQL
$Hg^{2+} + HCO_3^- = HgHCO_3^+$	16.3	MINEQL
$Hg^{2+} + EDTA^{4-} = HgEDTA^{2-}$	23.5	Stumm and Morgan 1996
$Hg^{2+} + EDTA^{4-} + H^+ = HgHEDTA^-$	27.0	Stumm and Morgan 1996
$Hg^{2+} + 2HS^- = Hg(SH)_2^0$	37.7	Benoit et al. 1999b
$Hg^{2+} + 2HS^- = HgS_2H^- + H^+$	31.5	Benoit et al. 1999b
$Hg^{2+} + 2HS^- = HgS_2^{2-} + 2H^+$	23.2	Benoit et al. 1999b
$Hg^{2+} + HS^- = HgSH^+$	30.2	Benoit et al. 1999b
$Hg^{2+} + HS^- + H_2O = HOHgSH^0 + H^+$	26.7	Benoit et al. 1999b
$Hg^{2+} + RS^- = HgRS^-$	31.1-32.2*	Skyllberg et al. 2000
$Hg^{2+} + RS^- = HgRS^-$	22.4-23.8*	Benoit et al. 2001a
$Hg^{2+} + RS^- = HgRS^-$	28.5*	Haitzer et al. 2002
$Hg^{2+} + RS^- = HgRS^-$	25.8-27.2*	Drexel et al. 2002
$RSH = RS^- + H^+$	-10	Benoit et al. 1999b
$MeHg^+ + OH^- = MeHgOH$	2.37	Stumm and Morgan 1996
$MeHg^+ + Cl^- = MeHgCl$	5.25	Stumm and Morgan 1996
$MeHg^+ + CO_3^{2-} = MeHgCO_3^-$	6.1	Stumm and Morgan 1996
$MeHg^+ + SO_4^{2-} = MeHgSO_4^-$	0.91	Stumm and Morgan 1996
$MeHg^+ + S^{2-} = MeHgS^-$	21.02	Stumm and Morgan 1996
$MeHg^+ + RSH = MeHgSR + H^+$	16.0-16.7	Karlsson and Skyllberg 2003
$MeHg^+ + RSH = MeHgSR + H^+$	16.7-17.1	Qian et al. 2002
$MeHg^+ + RSH = MeHgSR + H^+$	14.5-15.0**	Amirbahman et al. 2002

* The complexation constants for Hg and MeH to DOM ($HgRS^-$ and MeHgSR) are expressed in terms of the reduced thiol concentration on the organic matter
** Reported constants for the reaction of MeHg with reduced sulfur groups with an acidity constant of 10^{-10}

Chapter 2: Influence of organic matter on the sorption of mercury and methylmercury to amorphous iron oxide and sulfide

2.1. Introduction

Mercury has a high affinity for particles, thus cycling of particles controls the distribution of mercury in aquatic systems. The association of Hg with particles results in its deposition to the sediment, the major repository for Hg in aquatic systems (Benoit et al. 1998). The sediment is often the zone in the aquatic systems where the majority of the bacterial conversion of Hg to MeHg, the form of mercury which bioaccumulates in aquatic organisms, occurs as a result of the dynamic redox process occurring in these regions. The distribution of Hg between the particle and dissolved phase is also important in Hg methylation because only dissolved phase Hg is available for methylation (Benoit et al. 2003). As a result of the redox cycling of iron (Fe) solids, Fe oxides and Fe sulfides have been cited as important solid phases controlling the partitioning and mobility of Hg and MeHg in aquatic systems (Iverfeldt 1988; Gobeil and Cossa 1993; Mason et al. 1993; Gagnon et al. 1996; Quemerais et al. 1998; Bloom et al. 1999a) but the relative importance of Fe solids and organic matter in Hg and MeHg partitioning has not been examined.

Under different environmental conditions, Fe solids and organic particles have been cited as important solid phases in the partitioning of Hg and MeHg onto particles (Bloom et al. 1999a; Mason et al. 1999a; Lawson et al. 2001b; Heyes et al.

2004; Turner et al. 2004b). The cycling of Fe could therefore, strongly influence the partitioning of Hg and MeHg in the sediment. Under oxic conditions, Fe(III) exists as solid oxides, however at the redox interface Fe(III) is reduced to Fe(II), which causes dissolution of the solid Fe oxides and a release of Fe into the dissolved phase. However, as sulfide increases deeper in the sediments, as a result of sulfate reduction, Fe-sulfide minerals form, resulting in a decrease in the dissolved Fe concentration (Stumm and Morgan 1996).

In some systems, Fe cycling has been proposed as a mechanism to explain the observed and assumed mobility of dissolved Hg and MeHg in both the water column (Iverfeldt 1988; Mason et al. 1993; Quemerais et al. 1998) and sediment (Gobeil and Cossa 1993; Gagnon et al. 1996; Bloom et al. 1999a). In several systems, profiles of dissolved MeHg in sediment porewaters appear to mimic the dissolved Fe profiles, leading to the hypothesis that Fe oxide and Fe sulfide are important in the partitioning of MeHg between the solid and dissolved phases. It has been suggested that Fe oxides in surface sediments act as a barrier preventing the diffusion of MeHg into the water column (Gagnon et al. 1996; Bloom et al. 1999). The relationship between Fe and Hg is not as strong as the observed relationship between MeHg and Fe (Gobeil and Cossa 1993; Heyes et al. 2004) and it should be noted that the relationship between Fe cycling and Hg or MeHg partitioning does not hold true in all systems (Covelli et al. 1999; Mikac et al. 1999). Also, while correlations between Fe cycling and Hg and MeHg partitioning has been observed, there is no way to determine from these studies if Hg and MeHg are binding directly to Fe solids in the environment. Redox cycling can also influence other processes, such as the partitioning of organic

matter between the solid and dissolved phase, which could be driving the observed correlations between Fe and Hg cycling.

While there is only circumstantial evidence that Fe cycling in natural systems plays a role in Hg and MeHg partitioning, it is well known that Hg has a high complexation affinity for Fe oxides (Dzombak and Morel 1990; Gunneriusson et al. 1995; Tiffreau et al. 1995; Collins et al. 1999). Surface complexation models, in which the sorption of Hg to Fe oxides is explained as an interaction of Hg with surface hydroxyl groups on the Fe oxides, have been used to describe the affinity of Hg to Fe oxides (Dzombak and Morel 1990; Gunneriusson et al. 1995; Tiffreau et al. 1995). The complexation of MeHg to Fe oxide has not been studied as extensively as Hg, but in the one study that examined the complexation of MeHg to goethite, it was determined that MeHg forms a one-to-one complex with surface hydroxyl groups (Gunneriusson et al. 1995).

Even though Hg has a strong affinity for Fe oxide, in natural environments the association of Hg and MeHg with Fe oxide might not occur as a result of a direct interaction of the metal with Fe oxide. It has been suggested that the binding of Hg and MeHg to Fe oxides in the environment is due to ternary complex formation between Hg or MeHg, organic matter, and Fe oxide (Regnell et al. 1997). Using EXAFS, ternary complexes have been observed between Hg, Fe oxide and inorganic ligands, such as chloride and sulfate (Kim et al. 2004). The influence of DOM on the sorption of other trace metals to Fe oxides depends on the metals relative affinity to DOM and Fe oxide. Organic matter has been shown to decrease the complexation of copper to oxide surfaces above pH 6 as a result the stronger affinity of copper for

dissolved organic matter (DOM) relative to hydroxyl surfaces (Davis 1984; Christl and Kretzschmar 2001). In contrast, the adsorption of cadmium to oxide surfaces is not as strongly affected by DOM as a result of the weak affinity of cadmium for organic matter (Davis 1984). Under oxic conditions, DOM ligands are the most significant complexers of Hg and MeHg in the dissolved phase (Ravichandran 2004). Therefore, the effect of organic matter on Hg and MeHg complexation with Fe oxides cannot be ignored. Organic matter can also bind to surface sites on Fe oxide (Mayer 1999) which could potentially change the interaction of Hg with these sites.

The interaction of Hg and MeHg with Fe sulfide has not been studied to the same extent as the interaction of Hg with Fe oxide. Under anoxic conditions, it has been speculated that Fe sulfide minerals and organic matter are likely the dominant phases responsible for the partitioning of Hg to sedimentary phases (Benoit et al. 1999b). Many trace metals have been shown to associated with pyrite (FeS_2), the dominate sedimentary Fe sulfide mineral, through both coprecipitation and adsorption (Kornicker and Morse 1991; Huerta-Diaz and Morse 1992; Morse 1994). Hg has a high degree of trace metal pyritization (DTMP), a measure of the amount of a trace metal associated with pyrite relative to the amount of the trace metal associated with reactive phases, such as amorphous Fe sulfide and oxides, suggesting that Hg has a strong affinity for pyrite. Mercury sediment partitioning models, driven by the dissolved phase Hg concentration, indicate that in most environments, Hg is sorbed onto reduced sulfur containing solid phases such as Fe sulfides or organic matter in the sediment (Benoit et al. 1999b).

While pyrite is often the most dominant Fe-sulfide mineral found in the sediment, acid volatile sulfides (AVS), such as amorphous Fe sulfide, mackinawite and greigite, can comprise as much as 50% of the Fe-sulfide solids in the sediment (Morse et al. 1987a). Relative to pyrite, metastable Fe sulfide phases undergo more rapid oxidation (Morse 1991; Zhuang et al. 1994) and therefore metals associated with AVS phases are more likely to be released from the solid phase during oxidative conditions compared to metals associated with pyrite. As a result, the interaction of Hg with AVS could have important implications for Hg methylation, since high Hg methylation rates are often found in the active redox cycling zone. Mackinawite has been shown to sorb and incorporate Co, Mn and Ni and for these metals, it was found that the smaller the solubility product (K_{sp}) of the solid metal sulfide, the stronger the affinity of the metal to mackinawite (Arakaki and Morse 1993). While Hg was not examined, it would be predicted to have a very strong affinity to mackinawite since the K_{sp} for cinnabar, a mercuric sulfide mineral, ($K_{sp} = 10^{-53.3}$) is smaller than any of the metals examined (Morel and Hering 1993; Morse and Arakaki 1993).

In this study we examined the interaction of Hg and MeHg with hydrous ferric oxide (HFO) and amorphous Fe sulfide, both in the presence and absence of dissolved organic matter. As a result of the amorphous nature of both of these solids, they likely represent the inorganic phases in aquatic systems which initially form and are most susceptible to redox changes. Therefore, they could have significant roles in the partitioning and mobility of Hg and MeHg throughout aquatic systems. The interaction of Hg with HFO has previously been examined (Dzombak and Morel 1990; Gunneriusson et al. 1995; Tiffreau et al. 1995), but most studies have assumed

that Hg is only interacting with one surface site on the HFO. In light of recent EXAFS data showing the interaction of Hg with two surface sites on goethite (Collins et al. 1999), the interaction of Hg with HFO needed to be reexamined. In natural systems it is likely that Fe oxide surfaces are coated with organic matter (Mayer 1999) and therefore it is also important to examine the interaction of Hg and MeHg with HFO in the presence of organic matter. While field collected data suggests that Hg interacts with Fe sulfide solids (Huerta-Diaz and Morse 1992) the strength of this interaction has not been measured. Additionally, there is currently in no data on the impact of organic matter on the interaction of Hg with Fe sulfide solids or on the interaction of MeHg with Fe sulfide solids.

2.2. Methods

2.2.1. Mercury and methylmercury complexation to hydrous ferric oxide

Hydrous ferric oxide (HFO), also known as amorphous ferric hydroxide and amorphous iron oxyhydroxide, was synthesized by adding $Fe(NO_3)_3$ to a 0.1 M $NaNO_3$ solution followed by the addition of sodium hydroxide to adjust the solution to pH 7 (Patterson et al. 1997). Sodium carbonate (0.1 M) was also added to the solution to help control the pH. Since HFO undergoes rapid structural changes in the first hour, the HFO solution was equilibrated for one hour before mercury was added and the pH was readjusted using sodium hydroxide and nitric acid (Dzombak and Morel 1990). After the mercury was added, the solution was allowed to equilibrate for 3 hours. Synthesis of HFO, using this method, results in a completely amorphous solid with a chemical formula of $Fe_2O_3*nH_2O$ with n ranging from 1 to 3 (Patterson

et al. 1997). Longer aging of the amorphous solid results in the formation of a more crystalline iron oxide solid such as goethite. The amorphous solid used in this study has a structure similar to ferrihydrite, a naturally occurring Fe oxide (Dzombak and Morel 1990).

After the initial 1 hour aging of the HFO, Hg or MeHg was added to the HFO solution and allowed to equilibrate for 3 hours. For experiments examining the interaction of Hg with HFO over a pH range, 15 mg Fe/L HFO solutions were used and 5 µg/L Hg was added to these solutions. A more concentrated HFO solution was used, 200 mg Fe/L, when the interaction of MeHg with HFO was examined and for these experiments 50 µg/L MeHg was added to the HFO solution. In order to investigate the influence of DOM on the sorption of Hg and MeHg to the HFO, DOM was added to the HFO solution at the same time as the Hg or MeHg. For these experiments, 15 mg Fe/L HFO and 50 µg/L Hg or MeHg solutions were used. A hydrophilic DOM isolate from the Florida Everglades (F1-HPoA) was used for all DOM experiments. Properties of this DOM isolate are shown in Table 2.1. The complexation constant of Hg to the DOM isolate has been previously determined (Benoit et al. 2001a).

After the sample solutions equilibrated for 3 hours, the solid and aqueous phases were separated using vacuum filtration through a quartz fiber filter. The solid phase was dissolved in a 3% nitric acid solution, and the aqueous phase was acidified to 3% with nitric acid. The concentration of Hg and Fe was determined in both fractions using a Hewlett Packard 4500 series inductively coupled plasma-mass spectrometer (ICP-MS). Since both the Fe and Hg concentrations were large,

Table 2.1: Characteristics of DOM isolates from the Suwannee River and the Florida Everglades used in the laboratory prepared solutions.

DOM	Isolation method	Average molecular weight	%C	%S	Reduced S (% of total S)	Carboxyl (mol kg^{-1} C)	Aromatic C (NMR) (%)
[1] Suwannee River NOM	Reverse Osmosis	nr	48.8	0.6	nr	9.85	nr
[2]F1-HPoA	XAD resin	1031	52.2	1.73	22.1	10.43	18.2

nr not reported
[1] Data for the Suwannee River NOM was obtained from the International Humic Substances Society
[2] F1-HPoA is a hydrophilic acid isolated from within Water Conservation Area 2A of the Florida Everglades and was provided by Dr. George Aiken, USGS Boulder, CO. Characteristics of this isolate were reported in Ravichandran (1999)

preconcentration of the Hg was not required and therefore, direct analysis of the samples, using an autosampler, was conducted. The concentration of Hg was determined from two Hg isotopes (Hg-200 and Hg-202). Bromine monochloride and aqueous gold was added to the solutions to reduce the adsorption of Hg to the various parts of the ICP-MS. The concentration of Fe was determined for Fe-54 and Fe-58. Interferences, as a result of the formation of argon oxides, can occur with the analysis of Fe-56, the most abundant isotope of Fe and with Fe-57. The argon interference does not affect the analysis at mass 58 and 54, so measurements were made with these isotopes. The low abundance of Fe-58 did not affect the analysis since the concentrations of Fe in the solutions were high.

2.2.2. Interaction of mercury and methylmercury with amorphous iron sulfide

Amorphous iron sulfide ($FeS_{(s)}$), also referred to as disordered mackinawite, was prepared using a previously described method (Patterson et al. 1997) in which

precipitation of amorphous $FeS_{(s)}$ was carried out by simultaneously adding 5 mL each of 1.0 M sodium sulfide and 1.0 M ferrous chloride to 30 mL of 0.1M NaCl. All solutions were prepared in an anaerobic chamber using deoxygenated 0.1 M NaCl. Once the $FeS_{(s)}$ was synthesized, it was rinsed with deoxygenated phosphate buffer (0.04M, pH 6 prepared in 0.1 M NaCl) three times by centrifuging the solution and then pouring off the supernatant. After each rinse, more buffer solution was added to the solid and the solid was resuspended into the solution using a vortex mixer. Using X-ray diffractometry, is has been shown that $FeS_{(s)}$ produced using this method was dominantly amorphous with traces of mackinawite (Patterson et al. 1997).

After the $FeS_{(s)}$ was synthesized and rinsed, the solid was diluted to create a 50 mg Fe/L solution. The dilution was done with a pH 6, 0.04 M phosphate buffer prepared in a deoxygenated 0.1 M NaCl solution. The 50 mg Fe/L $FeS_{(s)}$ solution was prepared using freshly prepared $FeS_{(s)}$ for each experiment. In order to examine the adsorption of Hg to amorphous $FeS_{(s)}$, a stable isotope, Hg-199, was added to the $FeS_{(s)}$ solution to obtain a concentration of approximately 0.5 µg/L Hg-199. The enriched Hg-199 stable isotope (91.95% purity) used in these experiments was purchased from Oak Ridge National Laboratories as solid HgO and dissolved in a dilute nitric acid solution. The Hg-199 was in the form of Hg^{199}-chloride when it was added to the $FeS_{(s)}$ solution. After the Hg was spiked into the $FeS_{(s)}$ solution, 25 mL aliquots were partitioned into 50 mL polyethylene terephthalate (PET) centrifuge tubes and shaken on an orbital rotator for 5 hours. Triplicate samples were collected to determine the total amount of Hg in the $FeS_{(s)}$ solution and triplicate samples were also collected to determine the amount of Hg associated with the solid and aqueous

phase. After shaking for 5 hours, the solid phase was collected on a glass fiber filter (GFF) and was subsequently dissolved in 20 mL of a bromine monochloride solution (32mM KBr, 15 mM $KBrO_3$ in 20 % HCl). Bromine monochloride was also added to the filtrate, resulting in a solution containing 15 mM KBr and 2 mM $KBrO_3$ in 9% HCl, and to the unfiltered samples (32 mM KBr , 15 mM $KBrO_3$ in 20% HCl). The bromine monochloride oxidized the sulfide in the solution which if not removed interfered with the Hg analysis. The processing of the samples was done in an anaerobic chamber up until the bromination step. After bromination samples were held for at least 24 hours before analysis to ensure the oxidation of the sulfide.

Samples were prepared as described above with the addition of Suwannee River natural organic matter (purchased from the International Humic Substances Society) to examine the influence of DOM on the adsorbance of Hg to amorphous $FeS_{(s)}$ at pH 6. The characteristics of this DOM isolate are shown in Table 2.1. The DOM was added to the $FeS_{(s)}$ solution at the same time as the Hg spike to obtain a concentration of 26 mg C/L. Similar experiments were conducted with MeHg to look at the sorption of MeHg to amorphous $FeS_{(s)}$ both in the absence and presence of DOM. For these experiments, MeHg-199 was added to the solutions to reach an approximate concentration of 0.7 µg/L. The MeHg-199 was synthesized from Hg-199 using methylcobalamin, using a slight modification to the method described in Hintelmann and Ogrinc (2003). The modification included the use of methylene chloride to extract the MeHg rather than toluene.

Total Hg analysis was done using standard Hg analytical methods (Gill and Fitzgerald 1987; Bloom and Fitzgerald 1988). As described above, bromine

monochloride was added to all solutions in order to oxidize organic matter and sulfide. Hydroxylamine hydrochloride was then added to the samples to reduce any excess bromine monochloride. The Hg in the samples was reduced using stannous chloride immediately prior to purging and amalgamation onto traps containing gold beads. The traps were then heated and the pulse of Hg released was detected using a Hewlett Packard 4500 inductively-coupled plasma mass spectrometer (Hintelmann and Evans 1997b; Hintelmann and Ogrinc 2003). Removal of sulfide from the solutions was extremely important since it interferes with the reduction and trapping of the Hg onto the gold columns. Hg-202 was used to determine the background concentration of Hg in all samples and Hg-199 was used as an isotope spike in amended samples. The calculations required to determine the amount of spike in each sample, as a result of impurities in the isotopic spike, are present in detail elsewhere (Hintelmann and Ogrinc 2003). Since a stable isotope of MeHg was used in the experiments, the background concentration of Hg and MeHg was not important and therefore the MeHg could be analyzed using total Hg techniques. Any Hg-199 above the background Hg level, as determined from Hg-202, was assumed to be MeHg-199. Stable isotopes of Hg and MeHg have been used in several studies in our laboratory (Heyes et al. 2004; Heyes et al. in press; Kim et al. in press; Sunderland et al. in press; Whalin and Mason in press). The concentration of stable isotope added to the samples in this study was well above the detection limit (10 pg/L) for this technique. A solid state ion selective electrode and a reference electrode (Thermo Electron Corporation) was used to analyze for sulfide (Eaton 1998). Sample and

standards were preserved in sulfide antioxidant buffer (SAOB) and analyzed within 4 hours.

2.2.3. Thermodynamic speciation calculations

Speciation calculations were performed using the program MINEQL+ (Environmental Research Software, Hallowell, ME, USA). The formation constants for Hg-sulfide complexes (Benoit et al. 1999b) and a complexation constant for Hg to the DOM isolate used in the HFO experiments (Benoit et al. 2001a), were added into the MINEQL+ database. The complexation constants for MeHg with hydroxide, chloride, carbonate, sulfide (Stumm and Morgan 1996) and DOM (Karlsson and Skyllberg 2003) were also added to the data base. All relevant constants are shown in Table 1.1.

2.3. Results

2.3.1. Mercury and methylmercury association with hydrous ferric oxide

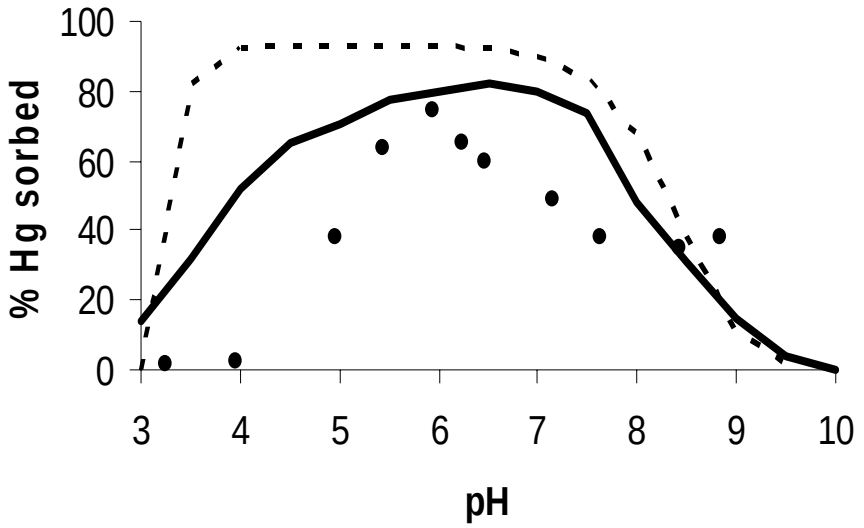
As found in other studies, the complexation of Hg with HFO is a function of pH (Figure 2.1a). The association of Hg with the HFO is affected by both the dissolved phase speciation of the Hg (Figure 2.2a) and the surface hydroxyl group protenation on the HFO (Figure 2.3). The surface hydroxyl groups are amphoteric (Equation 1 and 2) (Dzombak and Morel 1990) and this contributes to the pH dependence of the sorption of Hg to HFO.

$$\equiv FeOH + H^+ = \equiv FeOH_2^+ \qquad \log K = 7.29 \qquad (1)$$

$$\equiv FeOH = \equiv FeO^- + H^+ \qquad \log K = -8.93 \qquad (2)$$

Figure 2.1: a) Sorption of Hg to HFO over a pH gradient. Experimental data (circles), modeled sorption using constants from this study (solid line) and previously reported constants (dotted line) b) Sorption of MeHg to HFO over a pH gradient. Experimental data (circles) and modeled sorption using the constants determined in this study (solid line)

a)



b)

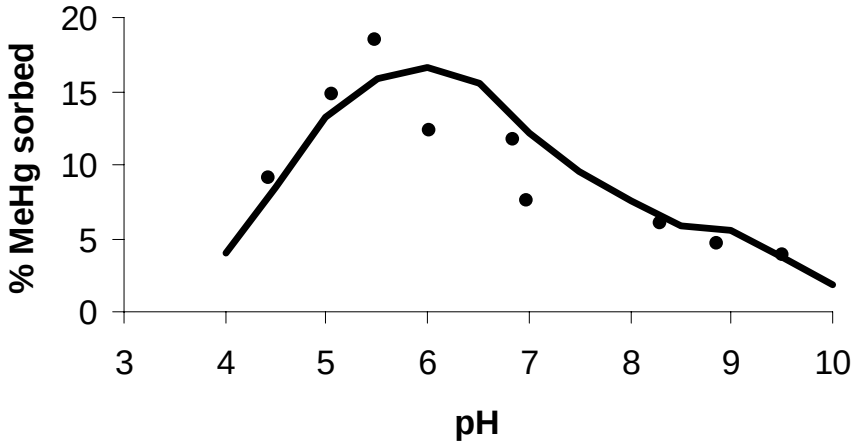
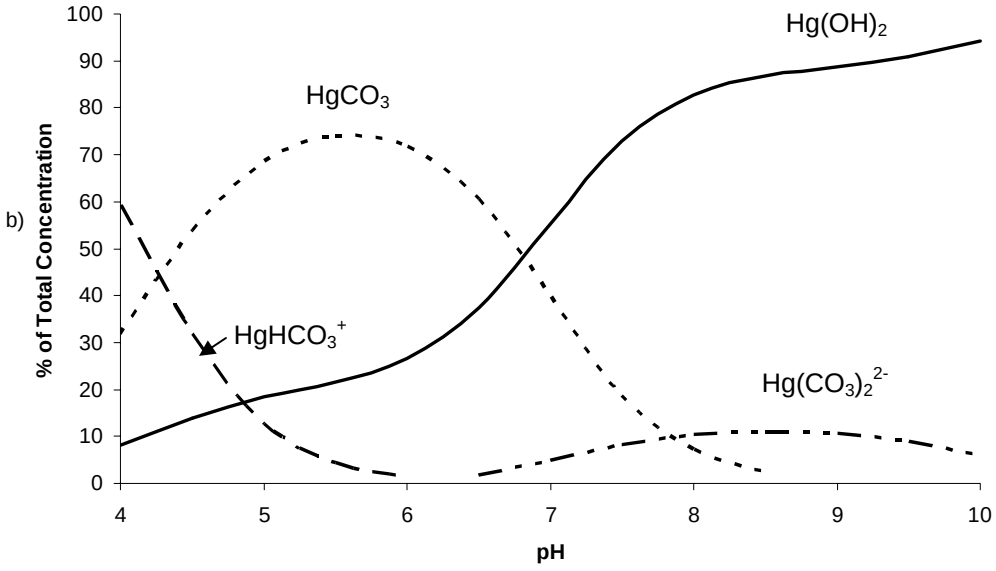


Figure 2.2: Dissolved phase speciation of Hg (a) and MeHg(b) in solution with HFO. Surface protenation state of HFO (c)

a)



b)

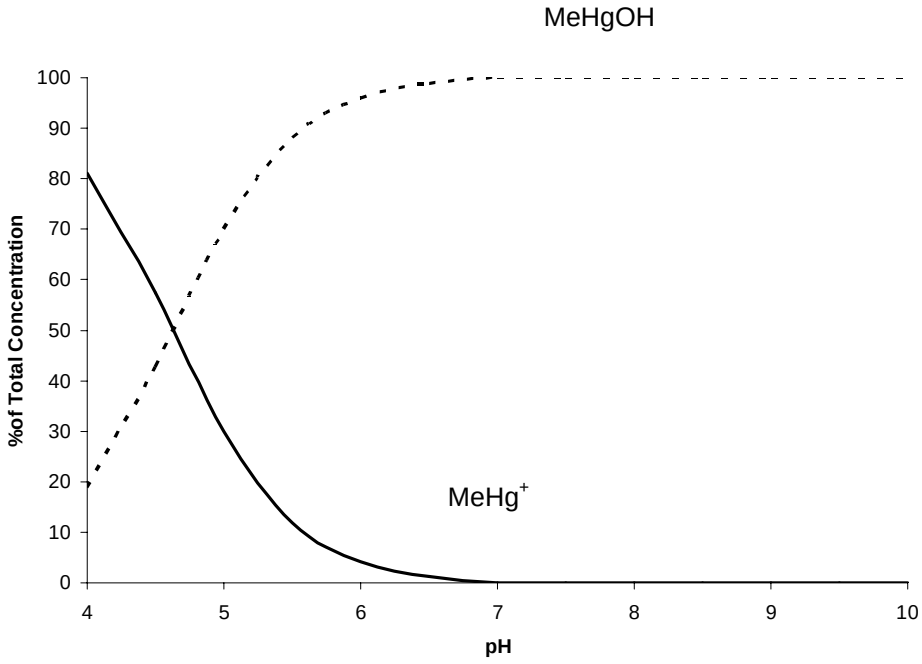
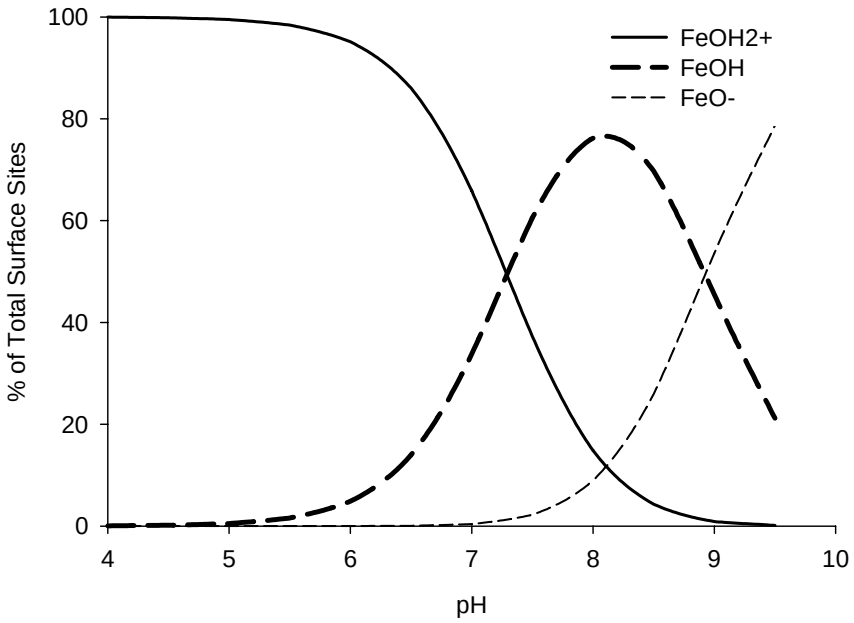


Figure 2.3: Surface site protenation of HFO over a pH gradient



Using the stability constants present in Table 1.1, the dominate dissolved phase species of Hg in the experimental solutions were determined to be $Hg(OH)_2$, $HgHCO_3^+$, $Hg(CO_3)_2^{2-}$, and $HgCO_3$ (Figure 2.2).

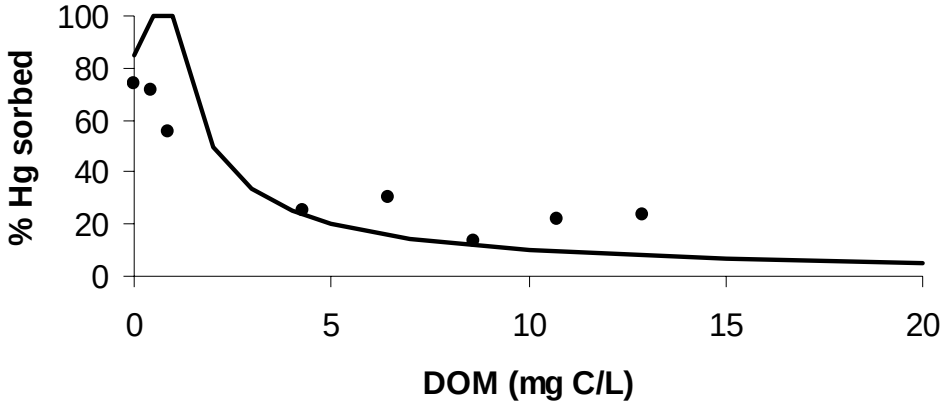
The influence of DOM on the adsorption of Hg to HFO was also examined over a range of DOM concentrations using a hydrophobic DOM isolate at pH 7. When DOM was not present, approximately 70% of the Hg was associated with the HFO but the addition of DOM resulted in a decreased association of the Hg with the HFO (Figure 2.3). Thermodynamic modeling, using MINEQL, indicates that at all of the DOM concentrations used, 100% of the dissolved phase Hg would be associated with the DOM as a result of the stronger interaction of Hg with DOM relative to the surface hydroxyl groups on the HFO.

The interaction of MeHg with HFO is pH dependent, as observed with the Hg, but the interaction is much weaker (Figure 2.1b). The dissolved phase speciation of MeHg is dominated by $MeHg^{+}$ and MeHgOH over the experimental pH range (Figure 2.3b). As with the Hg, the complexation of MeHg with the HFO is a function of the dissolved phase speciation of the MeHg and the protenation state of the HFO. A decrease in the association of MeHg with the addition of DOM was not observed as seen with the Hg (Figure 2.4b).

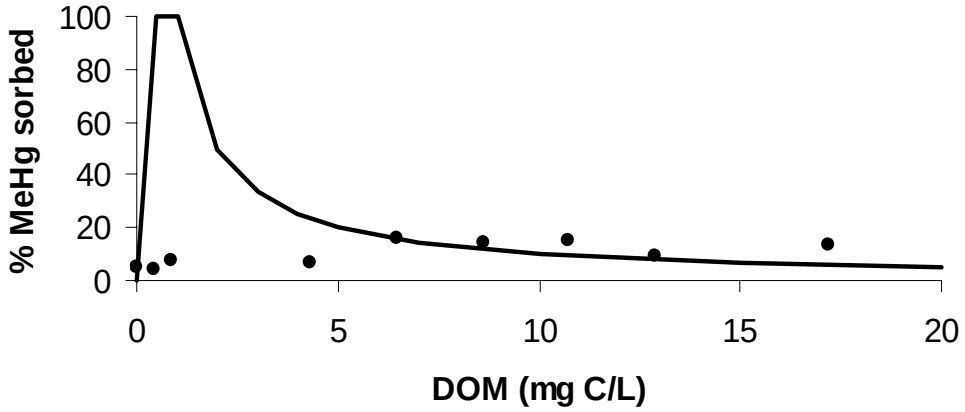
From the experimental data, and in order to compare the measured binding to the laboratory phase to environmental conditions, partitioning coefficients (K_d's) were determined for Hg and MeHg in the absence and presence of DOM. Partitioning coefficients measure the distribution of a metal between the particulate and dissolved phase. When DOM was not present, the log K_d's for Hg and MeHg, calculated from data collected at around pH 6, were 4.5 L kg^{-1} and 3.8 L kg^{-1} respectively. In order to determine the K_d's for the experiment solutions containing DOM, the data collected at DOM concentrations above 5 mg C/L was used. Under these conditions, approximately 20 % of the Hg and 15% of the MeHg was associated with the HFO resulting in log K_d's of 3.5 L kg^{-1} for Hg and 4.1 L kg^{-1} for MeHg. The K_d's for both Hg and MeHg in both the present and absence of DOM were within the range of log K_d's reported for natural sediment which range from 4.1-5.7 L kg^{-1} for Hg and 2.15-5.32 L kg^{-1} for MeHg (Bloom et al. 1999a; Mason et al. 1999a; Lawson et al. 2001b; Kim et al. 2004).

Figure 2.4: Experimental data (circles) and modeled (line) Hg (a) and MeHg (b) complexation to HFO in the presence of organic matter at pH 6.5

a)



b)



2.3.2. Complexation constants of mercury to hydrous ferric oxide

Conditional stability constants were calculated in order to examine the mechanism controlling the interaction of Hg with HFO in the presence and absence of DOM. Surface complexation models, in which the sorption of Hg to Fe oxides is explained as an interaction of Hg with surface hydroxyl groups on the Fe oxides, have been used to describe the affinity of Hg to Fe oxides (Dzombak and Morel 1990; Tiffreau et al. 1995). Most previous studies have assumed that Hg reacts with one surface hydroxyl group resulting in a loss of one proton (Equation 3). However, a study using EXAFS spectroscopy enabled the examination of the sorption mechanism of Hg to goethite, a Fe oxide mineral, which indicated that Hg associates with two surface hydroxyl groups. It was concluded that two protons are released in the sorption process (Equation 4), but under different experimental conditions, the protons could be retained by the Fe oxide-Hg complex (Equation 5). While complexation constants have been determined assuming Reaction 1 is occurring, (Dzombak and Morel 1990; Tiffreau et al. 1995) they have not been determined assuming that Equation 4 and 5 represent Hg binding over the pH range.

$$Hg^{2+} + \equiv FeOH \leftrightarrow \equiv FeOHg^{+} + H^{+} \quad (3)$$

$$Hg^{2+} + 2 \equiv FeOH \leftrightarrow (\equiv FeO)_2Hg + 2H^{+} \quad (4)$$

$$Hg^{2+} + 2 \equiv FeOH \leftrightarrow (\equiv FeOH)_2Hg^{2+} \quad (5)$$

Equations 3-5 are written as reactions of Hg with singly protenated surface sites of the HFO. It should be noted that this does not suggest that Hg is only reacting with

these surface sites. Figure 2.5 shows potential reaction mechanisms of Hg with the three types of surface sites, described by Equations 1 and 2 and shown in Figure 2.3.

In order to calculate complexation constants, it was assumed that only one type of cation binding sites on the HFO interacts with Hg, at a concentration of 0.029 mole sites/mol Fe (Tiffreau et al. 1993). Cation surface complexation models have also been developed that use two different types of binding sites, high and low affinity (Dzombak and Morel 1990), but it has been argued that published sorption data for Hg to HFO do not support this idea (Tiffreau et al. 1993). Also, at the low Hg concentrations used here, it is only the stronger sites that are likely to be involved in Hg complexation. Surface precipitation of Hg was not included in the calculations since the ratio of Hg to Fe was low in the experiments, as it is in most natural systems, therefore eliminating the presence of surface precipitation.

In order to determine the Hg complexation constant to HFO, it was assumed that Hg forms two complexes with HFO, $\equiv(FeO)_2Hg$ and $\equiv(FeOH)_2Hg^{2+}$ (Equations 4 and 5). The mass law expression for the formation of these complexes (Equation 6 and 7 respectively) were used to calculate the complexation constant for $\equiv(FeO)_2Hg$ ($K_{(FeO)2Hg}$) and $\equiv(FeOH)_2Hg^{2+}$ ($K_{\equiv(FeOH)2Hg2+}$).

$$[\equiv(FeO)_2Hg]\,[H^+]^2\,/[Hg^{2+}]\,[\equiv FeOH]^2 = K_{\equiv(FeO)2Hg} \quad (6)$$

$$[\equiv(FeOH)_2Hg^{2+}]\,/[Hg^{2+}]\,[\equiv FeOH]^2 = K_{\equiv(FeOH)2Hg2+} \quad (7)$$

For all complexation constants, the concentration of ≡FeOH (mole binding sites/L) was determined from the concentration of Hg binding sites (0.029 mole site per mole

Fe), taking into account the protenation state of the HFO at the different pH's. At pH values less than six, the surface hydroxyl sites of HFO are fully protenated ($\equiv FeOH_2^+$) (Figure 2.3) and therefore it was assumed that $\equiv(FeOH)_2Hg^{2+}$ dominated the complexation of Hg to HFO. Using experimental data collected below pH 6 (Figure 2.1) and Equation 7, log $K_{(FeOH)2Hg2+}$ was calculated. This constant and the conditional stability constant for $\equiv(FeO)_2Hg$ were optimized using the experimental data and MINEQL+. The log conditional stability constant for $\equiv(FeOH)_2Hg^{2+}$ and

Table 2.2: Complexation Constants for Hg and MeHg to HFO

Reaction	Log K
$Hg^{2+} + \equiv FeOH \leftrightarrow \equiv FeOHg^+ + H^+$	6.9[a], 7.76[b]
$Hg^{2+} + 2 \equiv FeOH \leftrightarrow (\equiv FeO)_2Hg + 2H^+$	4.3[c]
$Hg^{2+} + 2 \equiv FeOH \leftrightarrow (\equiv FeOH)_2Hg^{2+}$	20.3[c]
$HSRCOO^- + \equiv FeOH + H^+ \leftrightarrow \equiv FeOOCRSH + H_20$	0.04-1.8[d]
$Hg^{2+} + HSRCOO^- + \equiv FeOH \leftrightarrow \equiv FeOOCRSHg^+ + H_2O$	0.04-1.8[e]
$MeHg^+ + \equiv FeOH \leftrightarrow \equiv FeOHMeHg^+$	6.0[c]
$MeHg^+ + \equiv FeOH \leftrightarrow \equiv FeOMeHg$	-1.7[c]

Notes:
a Tiffreau et al. 1995
b Dzombak and Morel 1990
c This study
d Gu et al. 1994; Gu et al. 1995; Thimsen and Keil 1998; Van de Weerd et al. 1999; Zhou et al. 2001
e assumed to be the same as the complexation of DOM to HFO

$\equiv(FeO)_2Hg$ were determined to be 20.3 and 4.3 respectively. Table 2.2 summarizes previously determined complexation constants and the constants determined in this study.

The conditional complexation constants determined in this study do a reasonable job at replicating the trend in the experimental data of an increase in the association of Hg to the HFO with increasing pH followed by a decrease in the sorption after pH 6 (Figure 2.1). The calculated complexation constants predict the increase in adsorption at a lower pH than was observed in the data, which could be a result of the dissolution and reprecipitation of the HFO at low pH. The calculated constants also predict a decrease in sorption above pH 8 which was not observed in the data. The lack of fit of the model to the data suggests the potential presence of other complexes that are not currently included in the model calculations.

The complexation constants calculated in this study provide as good of a fit to the experimental data relative to previous reported complexation constants (Figure 2.1) (Dzombak and Morel 1990). In calculating the complexation constant, it was assumed that Hg is interacting with two surface sites on the HFO, an interaction which is supported by EXAFS data on the sorption of Hg to goethite. Previous studies did not consider this interaction while calculating the complexation constant of Hg to HFO. The deviation of the modeled sorption of Hg to HFO relative to the experimental data could be a result of multiple interactions occurring over the pH range examined (Figure 2.5). The EXAFS examination of Hg sorption to goethite was conducted at pH 4.6, therefore, it is possible that at higher pH values that Hg is forming a complex with only one surface site on the HFO. The formation of

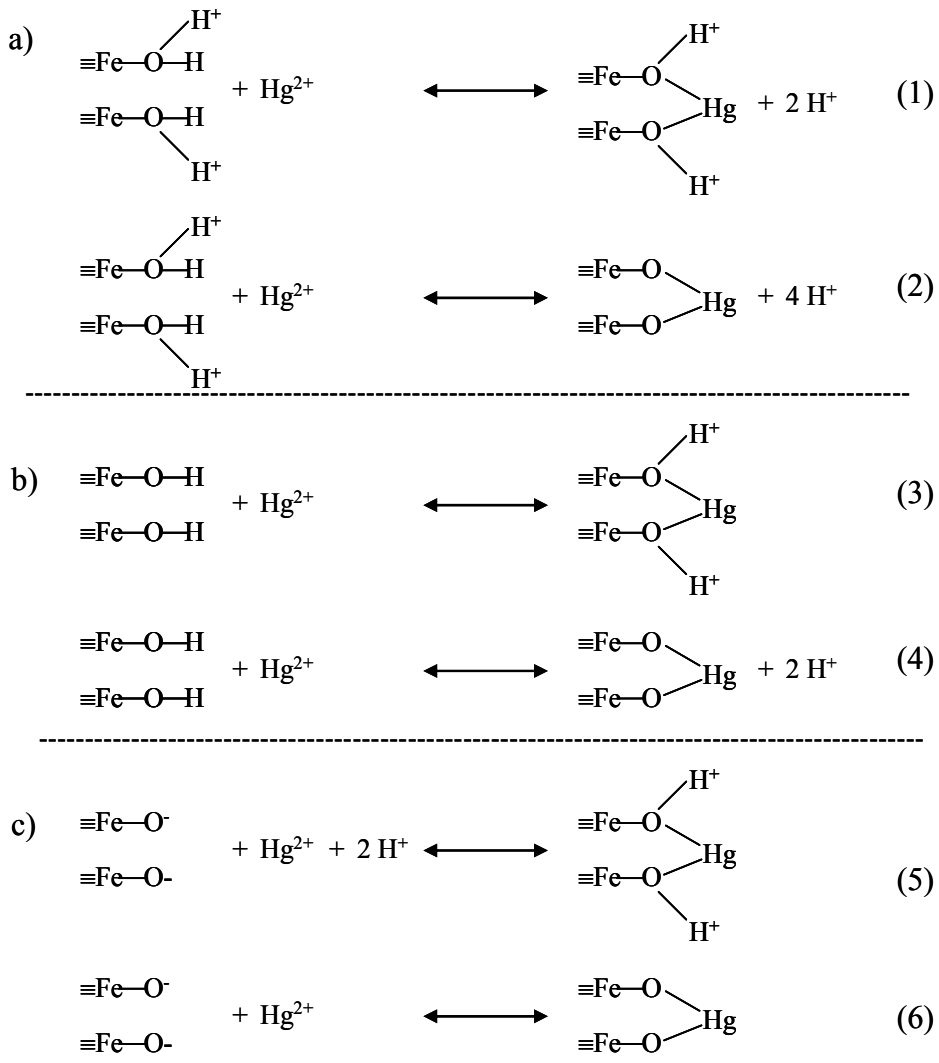


Figure 2.5: Potential reactions of Hg with surface hydroxyl groups on HFO. Reactions 1 and 2 involve the interaction of Hg with doubly protenated surface sites, the dominant sites below pH 6. Reactions 3 and 4 involve the interaction of Hg with neutral surface sites, which are the dominant surface sites between pH 7 and 9. The reactions of Hg with unprotenated surface sites, the dominant sites above pH 9 are depicted by Reactions 5 and 6.

≡FeOHgOH (Equation 8) could be important at higher pH where $Hg(OH)_2$ is the dominant dissolved Hg species. This type of interaction has been described for the sorption of Hg to goethite (Tiffreau et al. 1995). Overall, however, even though the conditional stability constants have a fair bit of uncertainty associated with them, and may not include all the potential interactions, they still provide a useful means to understand the association of Hg with the HFO in the presence of DOM, as described below. More studies are needed under a variety of conditions to examine under what scenarios single or dual complexes exist for Hg and HFO. The current model provides a reasonable estimation, especially given the results below that suggest that in most natural systems, direct association of Hg and HFO is not occurring.

$$Hg^{2+} + \equiv FeOH + H_2O \leftrightarrow \equiv FeOHgOH + 2H^+ \qquad (8)$$

It is obvious from the experimental data that DOM is changing the interaction of the Hg with the HFO (Figure 2.4a). Using the calculated conditional complexation constants for Hg sorbing to HFO, and the previously determined complexation constant for Hg to the DOM used in the experiments (Benoit et al. 2001a), thermodynamic modeling suggests that the Hg should be completely complexed to the DOM in solution at all the DOM concentrations used in this study. Therefore, the interaction of Hg with HFO in the presence of DOM can not be explained as a competitive ligand interaction between the DOM and surface hydroxyl groups on the HFO. In order to explain the association of Hg with HFO in the presence of DOM, the interaction of the DOM with the HFO also needs to be considered. The association of Hg with the HFO and subsequent decrease in its association with increasing DOM concentration can be explained by the formation of a ternary

complex between Hg, DOM and HFO (Equation 9). In this equation DOM is depicted as $HSRCOO^-$, where HS is a reduced sulfur functional group and COO^- represents a carboxylic acid group on the DOM.

$$Hg^{2+} + HSRCOO^- + \equiv FeOH \leftrightarrow \equiv FeOOCRSHg^+ + H_2O \qquad (9)$$

The interaction of DOM with Fe oxide surfaces has been well studied for a variety of DOM isolates (Tipping 1981; Tipping 1986; Gu et al. 1994; Gu et al. 1995; Gu et al. 1996a; Gu et al. 1996b; Meier et al. 1999; Zhou et al. 2001). Using previously measured DOM complexation constants, the complexation of DOM with HFO was modeled as an interaction between HFO surface hydroxyl groups and carboxylic acid functional groups on the DOM (Equation 10).

$$\equiv FeOH + HSRCOO^- \leftrightarrow \equiv FeOOCRSH + OH^- \qquad (10)$$

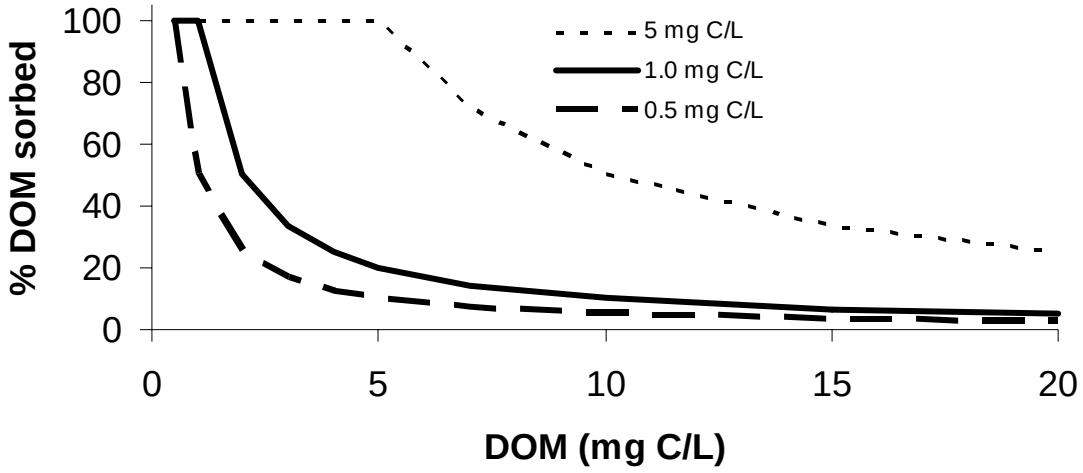
In several studies, the complexation constant of DOM to Fe oxide has been determined using isotherms for whole and fractionated DOM isolated from several locations (Gu et al. 1994; Gu et al. 1995; Thimsen and Keil 1998; Van de Weerd et al. 1999; Zhou et al. 2001). The complexation constants for DOM to HFO, written in terms of reaction 10, and determined from these studies, range from 0.9 to 60 L mg C^{-1}. The largest constants were determined for DOM fractions greater than 10 kDa. The constants for DOM not fractionated by size, range from 1.1 to 3.8 L mgC^{-1}.

The concentration of sites on the HFO available for the complexation of DOM was estimated using two separate methods. The first estimate was determined using a maximum adsorbed density of DOM to Fe oxide at near neutral pH values, which ranged from 0.166 to 0.285 mg C m^{-2} (Gu et al. 1994; Gu et al. 1995; Zhou et al. 2001). While these values were obtained using mineral forms of Fe oxide, they

provide an estimate of the concentration of surface sites available to complex DOM on HFO. The surface area of HFO have been estimated to be between 159 and 840 m^2 g Fe^{-1} , but 600 m^2 g Fe^{-1} has been suggested as the most accurate value (Dzombak and Morel 1990). Using the surface area of HFO and the sorption density of DOM on Fe oxide, the concentration of surface sites available to complex DOM was estimated to range from 2.1 to 3.6 mg C/L equivalents. The second estimate of surface sites available for the complexation of DOM by HFO used the assumption that DOM acts like an inorganic anion since DOM sorbs to surface hydroxyl sites through negatively charged carboxylic acid functional groups on the DOM. The concentration of available sites for anion sorption onto HFO has been measured as 0.2 mole sites per mol Fe (Dzombak and Morel 1990). Converting this abundance to mg C L^{-1} equivalents, the concentration of binding sites estimated using this method is 0.64 mg C L^{-1} equivalents.

The DOM concentration was expressed in mg C/L for the complexation calculations. Since the amount of Fe and the volume of the experimental solutions were constant throughout the experiment, expressing the DOM bound and the Fe surface site concentration as mg C L^{-1} equivalents does not change the complexation constants of DOM to HFO. The constant determined for a hydrophobic isolate from a wetland pond in Georgetown, SC (K= 3.81 L mgC^{-1}) (Gu et al. 1995) was used to model laboratory data since a hydrophobic DOM isolate was used in the experiment. Using the two different methods to estimate the concentration of sites available for the complexation of DOM on the HFO, the site concentration was determined to

Figure 2.6: Modeled sorption of DOM onto HFO at three surface group concentrations



range from 0.64 to 3.6 mg C L^{-1}equivalents. The sorption of DOM to HFO was, therefore, modeled using a range of site concentrations (Figure 2.6). As a result of the strong adsorption of DOM to HFO, 100% of the DOM in solution will be associated with the HFO until all of the surface sites on the HFO are covered. Changing the complexation constant of DOM to HFO several orders of magnitude does not influence the amount of DOM sorbed onto the surface of the HFO as a result of the strong affinity of DOM to HFO. The most sensitive parameter in the model was the concentration of binding sites on the HFO available to complex DOM.

In our attempt to model the interaction of Hg to HFO in the presence of DOM, it was assumed that the binding of DOM to HFO did not change when Hg or MeHg was complexed to the DOM, since different functional groups on the DOM are involved in the two reactions. The binding of DOM to HFO occurs through carboxylic functional groups on the DOM and surface hydroxyl groups on HFO

(Tipping 1986). The complexation of Hg to DOM occurs through reduced sulfur functional groups, such as thiols on DOM (Dyrssen and Wedborg 1991; Xia et al. 1999; Ravichandran 2004). It was also assumed that the complexation of Hg with DOM in the aqueous phase is similar to the complexation of Hg to DOM sorbed to HFO. Using these assumptions, the fraction of Hg associated with the HFO is equal to the fraction of DOM associated with the HFO since the Hg will be proportionately distributed between DOM on the HFO and DOM in the dissolved phase. The model only considers ternary complexes in which Hg-DOM complexes are bound to the HFO through an interaction of the DOM with the HFO (Equation 9).

In the experiments, since the surface site concentration ranged between 0.64 and 3.6 mg C L^{-1}equivalents, the surface sites were saturated with DOM below 5 mg C L^{-1} DOM. The addition of more DOM, therefore, results in an increasing dissolved phase concentration of DOM. Using the complexation constant of DOM to Fe oxide and the assumption that the Hg will interact with both DOM in the dissolved phase and that partitioned onto the HFO, the model predicts the decrease in Hg associated with the HFO with increasing DOM concentration (Figure 2.4a). In order to model the association of DOM with HFO, a surface site concentration available for DOM sorption of 1 mg C L^{-1} equivalents was used. The model provides a reasonable fit to the experimental data suggesting that the complexation of Hg with HFO in the experiments is being controlled by a ternary complex with the interaction between DOM and HFO driving the association of Hg to the HFO. Since the association of Hg to HFO is controlled by the sorption of DOM to the HFO, the most sensitive parameter in this modeling is the density of sites available to sorb DOM.

In another study, the influence of fulvic acid on the adsorption of Hg to goethite was examined and it was shown that the fulvic acid (20 mg C/L) increased the adsorption of Hg to the goethite (Backstrom et al. 2003), which initially appears to counter the results from our study. However, the goethite concentrations used by Backstrom et al. (5 g/L) were much larger than the concentration of HFO used in our study. They observed that at pH less than 7, over 80% of the fulvic acid adsorbed to the goethite. Information gained from our experiments suggests that the Hg will be associated with the goethite through an interaction of the Hg with fulvic acid sorbed onto the goethite surface, and since 80% or more of the fulvic acid is sorbed to the goethite, a majority of the Hg would also be associated with the goethite. Thus, these results are consistent with the proposed interaction described above.

2.3.3. Complexation constant of MeHg to HFO

The complexation of MeHg to Fe oxide has not been studied as extensively as Hg, but in the one study that examined the complexation of MeHg to goethite, it was determined that MeHg forms a one-to-one complex with surface hydroxyl groups (Gunneriusson et al. 1995). The hydrogen on the surface hydroxyl group can be retained (Equation 12) or lost (Equation 13) during the complexation. In order to determine the complexation constant of MeHg to HFO, at pH values less than six, it was assumed that only $\equiv FeOHMeHg^+$ formed and at higher pH values both, $\equiv FeOHMeHg^+$ and $\equiv FeOMeHg$ formed. From the experimental data, the conditional complexation constant for the formation of $\equiv FeOHMeHg^+$ was determined. Using the calculated constant, MINEQL+ was used to optimize this constant, along with

determining the constant for the formation of ≡FeOMeHg. The log conditional complexation constants for $\equiv FeOHMeHg^+$ and ≡FeOMeHg were 6.0 and -1.7 respectively (Figure 2.1b). The affinity of MeHg for HFO is much less than that of Hg.

$$MeHg^+ + \equiv FeOH \leftrightarrow \equiv FeOHMeHg^+ \quad (12)$$

$$MeHg^+ + \equiv FeOH \leftrightarrow \equiv FeOMeHg + H^+ \quad (13)$$

The complexation constant for reactions 12 and 13 (log K = 6.0 and –1.7 respectively) are similar to values determined by others (log K = 5.7 and –2.2) for MeHg binding to goethite (Gunneriusson et al. 1995). The higher complexation constants determined in our study is likely a result of the higher reactivity of surface groups on HFO compared to goethite.

The addition of DOM did not significantly increase the complexation of MeHg to HFO (Figure 2.4). As with Hg, it has been shown using X-ray absorption studies that MeHg complexes with reduced sulfur functional groups on organic matter (Qian et al. 2002; Yoon et al. 2005). The measured complexation constant of MeHg with DOM has been shown to support the interaction of MeHg with reduced sulfur functional groups on the DOM (Amirbahman et al. 2002; Qian et al. 2002; Karlsson and Skyllberg 2003). As is common with the complexation of MeHg to different ligands, the interaction of MeHg with DOM is weaker than the complexation of Hg with DOM (Dyrssen and Wedborg 1991). Since the interaction of MeHg with DOM is similar to the interaction of Hg with DOM, it would be expected that the interaction of MeHg and Hg with HFO in the presence of DOM would be similar. As with Hg, at all DOM concentrations used in the experiments, the dissolved phase speciation of

MeHg should be dominated by the MeHg-DOM complex. It was shown with Hg that the interaction of DOM with the HFO controlled the association of Hg to the HFO as a result of the formation of a ternary complex. If the same interaction was occurring with MeHg (Equation 17), an increased association of the MeHg would be expected at low DOM concentrations, which was not observed.

$$MeHg^{+} + HSRCOO^{-} + \equiv FeOH \leftrightarrow \equiv FeOOCRSMeHg + H_2O \qquad (17)$$

The addition of DOM appears to slightly increase the association of MeHg to the HFO at the higher DOM concentrations, but the interaction was different than what was observed with the Hg especially at low DOM concentrations. If the interaction of MeHg with HFO was stronger than the interaction of MeHg to DOM the experimental data could be explained by a competitive interaction between the HFO surface sites and the DOM. The measured complexation constants for the MeHg-DOM complex range over a couple of orders of magnitude (Amirbahman et al. 2002; Qian et al. 2002; Karlsson and Skyllberg 2003), but even using the lowest reported constants to model the interaction MeHg with HFO in the presence of DOM results in 100% of the MeHg complexing to the DOM. The complexation constant for the Hg-DOM complex would have to be approximately 5 orders of magnitude weaker in order for the direct complexation of MeHg to the HFO to be important. Therefore, this is not a likely explanation for the experimental data. While the mechanism controlling the interaction of MeHg, in the presence of DOM, with HFO appears to be different than the interaction of Hg to HFO in the presence of DOM, this interaction is not completely understood.

2.3.4. The interaction of mercury and methylmercury with amorphous iron sulfide

Both Hg and MeHg bind strongly to amorphous $FeS_{(s)}$. Greater than 99% of the added Hg was associated with the solid $FeS_{(s)}$ at pH 5.0, 5.9 and 8.1. Even in the presence of DOM, the adsorption of Hg to the $FeS_{(s)}$ was greater than 99% indicating that DOM is a weaker ligand compared to the surface groups of the $FeS_{(s)}$ and therefore did not hold the Hg in solution. Similar results were also found with MeHg in which greater than 99% of the MeHg was sorbed to the $FeS_{(s)}$ and in the presence of DOM, 98% MeHg sorbed. The sulfide concentration in the dissolved phase ranged from 10-100 μM with the variability occurring as a result of pH. Using the complexation constants shown in Table 1.1 and the measured pH and sulfide concentration in the dissolved phase of the experimental solutions, the speciation in these solutions was determined to be dominated by inorganic Hg-sulfide complexes ($Hg(SH)_2$, $HgHS_2^-$, HOHgSH). Since the concentration of Hg in the dissolved phase is below the intrinsic solubility of cinnabar ($\log K_{sp} = 53.3$) (Stumm and Morgan 1996), the association of Hg with the solid phase can not be a result of the precipitation of $HgS_{(s)}$.

Partitioning coefficients for Hg and MeHg in the experimental solutions were determined for the association with FeS, in both the presence and absence of DOM. The log K_d's were similar for all solution ($\log K_d = 6.3$-6.5) and were at least an order of magnitude higher than K_d's reported from natural samples. The amorphous $FeS_{(s)}$ used in the experiments is likely more reactive than most natural anoxic sedimentary phases, such as mackinawite or pyrite which are more structured solid phases. Under natural sedimentary conditions these other Fe-sulfide solid phase likely also

contribute to the sedimentary partitioning of Hg and MeHg resulting in lower K_d's in the environmental samples.

2.3.5. Complexation constant of mercury to amorphous iron sulfide

The complexation of divalent cations to Fe sulfides occurs through the interaction of the metal with surface sulfur groups on $FeS_{(s)}$ solids (Jean and Bancroft 1986; Kornicker and Morse 1991). It has been shown that amorphous $FeS_{(s)}$ contains two types of surface sites, a strongly acidic mono-coordinated surface functional sulfur group ($\equiv FeSH^0$) and a weakly acidic tricoordinated sulfur site ($\equiv Fe_3SH^0$). Both types of sites can undergo protenation reactions (Equations14-17) (Wolthers et al. 2003; Wolthers et al. 2005).

$$\equiv FeSH^0 + H^+ \leftrightarrow \equiv FeSH_2^+ \qquad \log K = 8.0 \pm 0.1 \qquad (14)$$

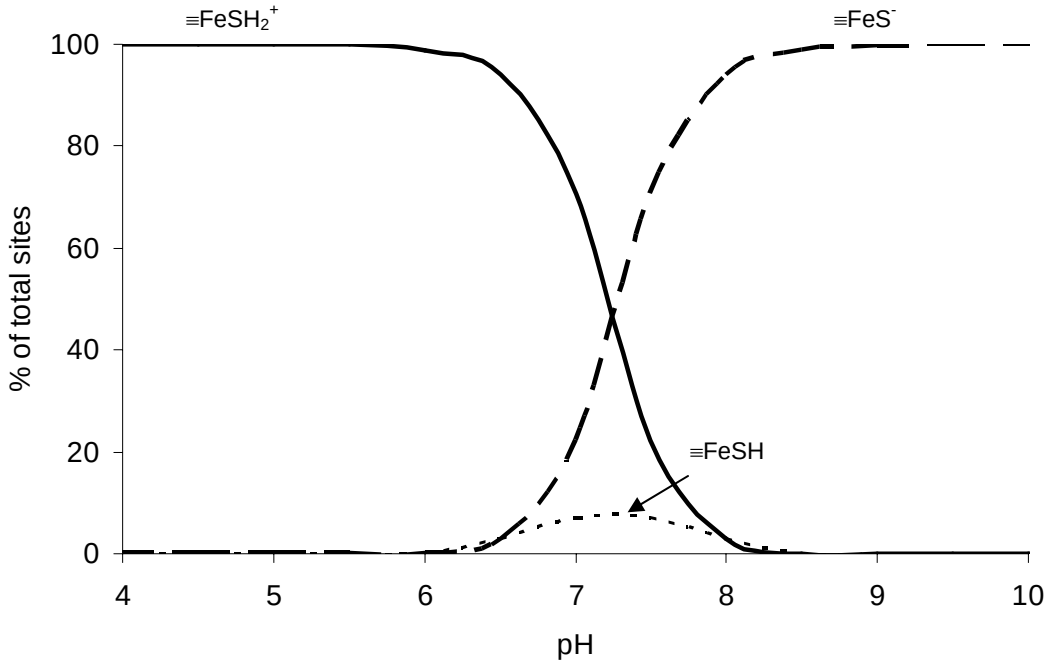
$$\equiv FeSH^0 \leftrightarrow \equiv FeS^- + H^+ \qquad \log K = -6.5 \pm 0.1 \qquad (15)$$

$$\equiv Fe_3SH^0 + H^+ \leftrightarrow \equiv Fe_3SH_2^+ \qquad \log K = 7.85 \pm 0.05 \qquad (16)$$

$$\equiv Fe_3SH^0 \leftrightarrow \equiv Fe_3S^- + H^+ \qquad \log K < -9.5 \qquad (17)$$

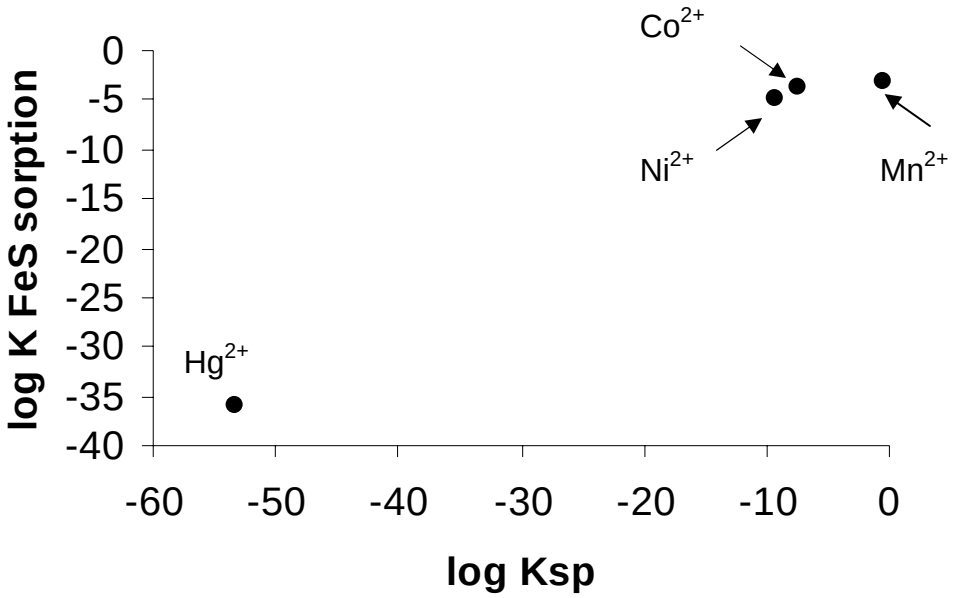
While the abundance of the two types of sites (1.2 mmol g^{-1} $FeS_{(s)}$) are the same, the mono-coordinated sites are stronger (Wolthers et al. 2003) and therefore the sites that are most likely involved in the complexation of Hg and MeHg. The complexation constant for Hg and MeHg to amorphous $FeS_{(s)}$ was determined using experimental data and Equations 18 and 19. The concentration of Hg^{2+}, which is low in the experimental solutions as a result of the dominance of dissolved Hg-sulfide complexes, was used to calculate the complexation of Hg to $FeS_{(s)}$. The concentration of the neutral surface sites on the $FeS_{(s)}$ was determined based on the surface

Figure 2.7: Surface site protenation state of amorphous FeS over a pH range



protenation reactions (Equation 14 and 15) (Figure 2.7) of the mono-coordinated sites and the total surface site abundance of these sites (1.2 mmol g^{-1} $FeS_{(s)}$) (Wolthers et al. 2003; Wolthers et al. 2005). Using these parameters, the complexation constant for Hg with amorphous $FeS_{(s)}$ was determined at three different pH's (Table 2.3) resulting in an average log K of 29.6 ± 0.6. The complexation constant for MeHg to amorphous Fe sulfide (Reaction 19) was also determined assuming that MeHg reacts with the sulfur groups on the $FeS_{(s)}$ solid (log K = 6.0). Previously it was shown that the complexation of several different trace metals to $FeS_{(s)}$ could be related to the solubility product of the metal sulfide solid, with larger sorption occurring for metals with lower solubility products. The log K for the complexation of Hg to amorphous $FeS_{(s)}$ and the solubility product of $HgS_{(s)}$ can be included on a plot with sorption

Figure 2.8: Relationship between the solubility product of several metal sulfide and the complexation constant of the metal onto amourphous FeS. Ni^{2+}, Co^{2+} and Mn^{2+} data from Morse and Arakaki (1993) and Hg data from this study



constants and solubility products of other trace metals (Figure 2.8) (Morse and Arakaki 1993). The complexation constant of Hg to $FeS_{(s)}$, measured in this study, fits the trend of increasing sorption with decreased solubility of the metal sulfide solid.

$$\equiv FeSH^0 + Hg^{2+} \leftrightarrow \equiv FeSHg^+ + H^+ \qquad \log K = 29.6 \qquad (18)$$

$$\equiv FeSH^0 + MeHg^+ \leftrightarrow \equiv FeSMeHg \qquad \log K = 6.0 \qquad (19)$$

The affinity of DOM to the surface sites on the amorphous $FeS_{(s)}$ has not been examined, but from this study it does not appear to influence the sorption of Hg to the amorphous $FeS_{(s)}$ and, therefore, a complexation constant for this interaction was not examined. Even if DOM was associated with the surface sites on the $FeS_{(s)}$, it would

not be expected for the Hg to interact with the DOM because of the much stronger interaction of Hg with reduced sulfur relative to DOM (Benoit et al. 2001a; Ravichandran 2004). Hg is more likely to react with uncomplexed surface sites or displace DOM from the surface sites to interact directly with the $FeS_{(s)}$. Since sulfide is present in the dissolved phase, the speciation of Hg should be dominated by Hg-sulfide complexes even in the presence of DOM.

Table 2.3: Conditional complexation constants for Hg and MeHg to amorphous FeS

Reaction	pH	Log K
$\equiv FeSH^0 + Hg^{2+} \leftrightarrow \equiv FeSHg^+$	**5.0**	**30.1**
	5.9	**28.9**
	8.1	**29.8**
	average	29.6 ± 0.6
$\equiv FeSH^0 + MeHg^+ \leftrightarrow \equiv FeSMeHg$	**6.2**	**6.0**

As a result of the strong interaction of MeHg with $FeS_{(s)}$ and dissolved sulfide, DOM did not influence the association of MeHg with $FeS_{(s)}$. Using the complexation constants in Table 1.1, the speciation of dissolved phase MeHg was dominated by MeHg-sulfide complexes as a result of the much stronger interaction of MeHg with sulfide relative to DOM. Speciation calculations indicate that the dissolved phase speciation of MeHg will be dominated by the MeHg-sulfide complex even in the

presence of DOM. Even if some of the DOM is associated with the $FeS_{(s)}$ it is unlikely that MeHg will be associated with the DOM on the surface as a result of the stronger interaction of MeHg with sulfide relative to DOM.

A comparison between the complexation strength of Hg to solid phase organic matter and amorphous $FeS_{(s)}$ can be made by comparing the complexation constants determined in this study for Hg to $FeS_{(s)}$ and known constants of Hg to organic matter. The complexation of Hg to organic matter occurs through the association of Hg with reduced thiol groups on the organic matter (Skyllberg et al. 2000; Benoit et al. 2001a; Haitzer et al. 2002; Ravichandran 2004). The most common reaction used to represent the complexation of Hg with reduced thiol groups on organic matter (Equation 20) involves the interaction of Hg with deprotenated thiol groups.

$$Hg^{2+} + RS^{-} \leftrightarrow RSHg^{+} \qquad \log K = 22.4\text{-}32.2 \qquad (20)$$

Complexation constants for Reaction 20 measured using both dissolved and particulate organic matter range from $10^{22.4}$ to $10^{32.2}$. In order to easily compare the complexation of Hg with organic matter and $FeS_{(s)}$, the complexation constant for Hg to the $FeS_{(s)}$ needs to be written as a reaction of Hg with a deprotenated surface sulfur group on the $FeS_{(s)}$ (Equation 21). The reaction of Hg with the neutral surface site of the $FeS_{(s)}$ (Equation 18) and the deprotenation reaction of the surface sulfur site on the $FeS_{(s)}$ (Equation 15) can be combined to obtain the complexation constant for Equation 21.

$$\equiv FeS^{-} + Hg^{2+} \leftrightarrow \equiv FeSHg^{+} \qquad \log K = 36.1 \qquad (21)$$

The complexation of Hg to deprotenated sulfur groups on $FeS_{(s)}$ is much stronger (log K = 36.1) than the complexation of Hg to organic matter (log K = 22.4-32.2). In

order to determine the importance of organic matter relative to Fe sulfide phases in the sediment for the complexation of Hg, the relative abundance of organic matter reduced thiol groups and $FeS_{(s)}$ surface sulfur groups needs to be known However, in order for organic matter to be important in the complexation of Hg, the abundance of reduced thiol groups of the organic matter would have to be several orders of magnitude higher than the abundance of surface sulfur groups on the $FeS_{(s)}$, which is not likely.

The importance of $FeS_{(s)}$ and solid phase organic matter in the complexation of MeHg can be examined in the same manor as described above for Hg. The complexation constant of MeHg to deprotenated sulfur sites on the $FeS_{(s)}$ (log K = 12.5) (Equation 22) is smaller than the complexation constant of MeHg to reduced sulfur groups on organic matter (log K = 14.5-17.1) (Equation 23) (Amirbahman et al. 2002; Qian et al. 2002; Karlsson and Skyllberg 2003). Unlike the scenario for Hg, the influence of $FeS_{(s)}$ might not be as important in the partitioning of MeHg to the solid phase as a result of the stronger interaction of MeHg with reduced thiol groups on partitculate organic matter.

$$\equiv FeS^- + MeHg^+ \leftrightarrow \equiv FeSMeHg \qquad \log K = 12.5 \qquad (22)$$

$$MeHg^+ + RS^- \leftrightarrow RSMeHg \qquad \log K = 14.5\text{-}17.1 \qquad (23)$$

2.4. Application of the results to natural system

The interaction of Hg and MeHg with particles such as Fe oxide, Fe sulfides and organic matter controls the mobility of Hg and MeHg within aquatic systems and the bioavailability of Hg to methylation. The distribution of Hg between the

particulate and dissolved phase has implications for the conversion of Hg to MeHg since it is thought that only dissolved phase Hg is available for bacterial uptake. While Fe oxides have been cited as important phases for the particle association of Hg under oxic conditions, the distribution of Hg on Fe oxide particles needs to include the interaction of Hg with organic matter, and organic matter with Fe oxides, to clearly understand the particle interaction of Hg under oxic conditions. The interaction of Hg with organic matter is stronger than the interaction of Hg with Fe oxide, resulting in organic matter playing a significant role in Hg partitioning.

In the environment, it has been shown that mineral surfaces are coated with a layer of organic matter. While the distribution of organic matter on minerals is patchy, resulting in uncoated mineral surfaces, iron oxide phases likely adsorb a large fraction of the organic matter as a result of the reactivity of these phases (Mayer 1999). The coating of Fe oxide with organic matter supports our result suggesting that Hg will be associated with organic matter rather than oxides in natural systems. This is further supported by a study which showed a decrease in the sorption of Hg to natural particles after the organic matter in the particles was oxidized (Turner et al. 2004b). Even though Hg is likely associated with organic matter, Fe cycling could impact the mobility of Hg at redox interfaces. As dissolution occurs, due to Fe reduction, organic matter associated with the Fe oxide could be released into the dissolved phase.

In this study and in previous studies, the interaction of Hg with Fe oxide has been used to try to understand the partitioning of Hg to sedimentary solids. While the interaction of Hg with Fe oxide is likely important in the particle association of Hg as

a result of the redox cycling of Fe, Fe minerals only constitute approximately 5% of the total inorganic solids in sediments [Shchulz, 2000 #347]. The influence of DOM on the interaction of Hg with Fe oxide described in this study can be applied to other inorganic solids, such as Mn oxide, quartz and clay minerals, which are potentially important in the association of Hg to particles. The association of Hg with these other inorganic solids is likely also controlled by the interaction of organic matter with the surfaces of these solids, and therefore, the surface areas of the solids are important. Fe oxides have a larger surface area (~159-234 m^2g^{-1}) in natural systems compared to clay minerals (30 m^2g^{-1}) and quartz (~5m^2g^{-1}) [Shchulz, 2000 #347;Tiffreau, 1995 #1] but as a result of the abundance of clay minerals and quartz in the sediment these inorganic solids could also be important in the partitioning of Hg. The association of Hg with Fe oxide is controlled by the association of DOM to the solid surface and it has been shown that DOM will sorb to the surfaces of different minerals to different extends [Davis, 1982 #346]. The sorption of DOM to inorganic surfaces is a function of the surface area of the inorganic solid and also the acidity of the surface functional groups on the solid. DOM interacts with positively charged surface hydroxyl groups on the inorganic solids but the acidity of the surface hydroxyl groups differs with different solids [Davis, 1982 #346]. For example, the point of zero charge for quartz is pH 2.5 (Davis 1982), so under natural conditions DOM will not react with quartz as strongly as it does with Fe oxide which has a of point of zero charge of around pH 8 (Dzombak and Morel 1990). While it is likely that Hg will associate to different inorganic solids as a result of the sorption of DOM to these solids, more work needs to be done to truly understand the nature of these interactions.

While organic matter has a significant impact on the complexation of Hg under oxic conditions, the interaction of Hg with organic matter is likely not as important under sulfidic conditions as a result of the strong interaction of Hg with $FeS_{(s)}$ and dissolved phase sulfide. The complexation of Hg to amorphous $FeS_{(s)}$ is much stronger than the association to reduced thiol groups on organic matter suggesting that $FeS_{(s)}$ is driving the partitioning of Hg between the solid and dissolved phase under sulfidic conditions. The complexation constants for MeHg to organic matter and $FeS_{(s)}$ are similar and, therefore, organic matter could be important in the partitioning of MeHg under anoxic conditions.

Chapter 3: Modification of octanol-water partitioning extractions to examine Hg-complexation in natural waters

3.1. Introduction

The complexation of inorganic mercury (Hg) has been shown to affect the mobility, transformation and bioaccumulation of Hg in the environment, but few methods are available to examine Hg complexation and speciation in natural waters. Competitive ligand exchange (Hsu and Sedlak 2003),"reducible Hg" titrations (Lamborg et al. 2003) and ultrafiltration (Babiarz et al. 2000; Choe et al. 2003) have been used to examine Hg complexation, mainly with dissolved organic matter (DOM). However, these techniques require the addition of Hg and have only been developed for oxic conditions. Further, while Hg-DOM complexes appear to be dominant in most oxic waters (except open ocean water) (Dyrssen and Wedborg 1991; Ravichandran 2004), it is neutral, inorganic Hg complexes that appear to be most important for uptake and bioaccumulation by microorganisms including phytoplankton (Mason et al. 1996a), Hg-methylating bacteria (Benoit et al. 2001c), and some but not all engineered Hg "bioreporter" bacteria (Barkay et al. 1997; Kelly et al. 2003). The measurement of these complexes in natural waters, including anoxic systems, is critical to understanding Hg methylation and bioaccumulation. To date, speciation determination has relied primarily on thermodynamic models because no analytical techniques are available to examine Hg complexation in unamended water samples. Under sulfidic conditions, thermodynamic models rely on complexation

constants determined using simple laboratory solutions and these predictions have never been validated in complex matrices such a natural water samples.

Octanol-water partitioning, a method often used to look at the hydrophobicity and bioaccumulation of organic contaminants, has also been used with trace metals (Faust 1992; Zhang et al. 1998; Reinfelder and Chang 1999; Benoit et al. 1999a; Fraser et al. 2000; Jay et al. 2000; Benoit et al. 2001a; Zimmermann et al. 2003; Turner and Mawji 2004a; Turner and Mawji 2005). While the partitioning of metals into octanol is small relative to the partitioning observed with many organic contaminants, differences in partitioning occur as a function of the complexation of trace metals with different ligands. For example, free silver ions (Ag^{+}) partition into octanol to a lesser extent than $AgCl^{0}$ as a result of the increased hydrophobicity of the neutral species (Reinfelder and Chang 1999). Octanol-water partitioning has been used to correlate metal speciation with the uptake of metals by algae (Reinfelder and Chang 1999), zooplankton (Mason et al. 1996a) and fish (Palmer et al. 1998). It has often been assumed that metal toxicity is a result of the free metal concentration but using octanol-water partitioning it has been shown that some small metal-organic complexes are more lipophilic than inorganic complexes (Palmer et al. 1998; Zhang et al. 1998; Zimmermann et al. 2003) and can be taken up by organisms (Palmer et al. 1998).

Several laboratory studies have employed octanol-water partitioning to examine Hg complexation and assess its impact on other processes such as Hg methylation and bioaccumulation (Halbach 1985; Mason et al. 1996a; Benoit et al. 1999a; Jay et al. 2000; Benoit et al. 2001a), but this technique has never been applied

to measure Hg complexation in natural water samples. In all previous studies, Hg concentrations well above those found in natural waters or a Hg radioisotope were used, and therefore detection limit problems and analytical interferences were not a substantial concern. In order to apply this technique to unamened natural samples, adjustments to the method were needed to reduce Hg contamination. Also, octanol interferes with Hg analysis when natural levels of Hg are being examined using cold vapor atomic fluorescence spectroscopy (CVAFS) and, therefore, modifications to the method were needed to remove residual octanol from the water phase. In this chapter, modifications to previously used octanol-water partitioning techniques are presented along with data showing the applicability and limitations of this technique to examine Hg complexation in natural samples.

3.2. Octanol-water partitioning theory

Octanol-water partitioning extractions involve the addition of octanol to a water sample in order to determine the fraction of Hg in the sample which will partition into octanol. Octanol-water partitioning coefficients (K_{ow}) are widely used to estimate the hydrophobicity of organic contaminants and the K_{ow} is considered a surrogate measure of the potential of a compound to partition into biological tissues. For Hg, neutral Hg species such as $HgCl_2^0$ or HgS^0 are more hydrophobic relative to charged Hg complexes and therefore partition to a greater extent into octanol. The partitioning coefficient of a pure Hg species (K_{ow}) is defined using Equation 1 where HgL_{oct} and HgL_{aq} are the concentrations of Hg bound to a specific ligand in the

Table 3.1: K_{ow} for several Hg complexes

Species	Kow	Species	Kow
$Hg(OH)_2^0$	1.2[a]	$HgSH^+$, HgS_2^{2-}	0*
$HgCl_2^0$	3.3[a]	HgS^0, $Hg(SH)_2$	72[c]
HgOHCl	0.05[a]	HgDOM	0.1[b], 1.7-3.3[c]
$HgCl^+$	0*		

* Charged complexes are assumed to not significantly partition into octanol (Benoit et al., 1999)
a Mason et al 1996
b Benoit et al 2001
c this study

octanol and aqueous phase, respectively. The octanol-water partitioning of several Hg complexes have previously been measured (Table 3.1).

When the octanol-water partitioning of Hg in a water sample is measured, an overall partitioning coefficient (D_{ow}) is determined since several Hg complexes, with different octanol solubilties, could be present. The D_{ow} is the ratio of the total Hg concentration in the octanol phase, Hg_{oct}, to the total Hg concentration in the aqueous phase, Hg_{aq} at the end of the extraction (Equation 2). The K_{ow} of individual Hg complexes ($(K_{ow})_i$) are related to the D_{ow} of a sample by the fraction of the individual complexes (α_i) present in the sample (Equation 3) (Faust 1992).

Equation 1: $K_{ow} = HgL_{oct}/HgL_{aq}$

Equation 2: $D_{ow} = Hg_{oct}/Hg_{aq}$

Equation 3: $D_{ow} = \sum \alpha_i (K_{ow})_i$

In previous studies using octanol-water partitioning to examine Hg, it has been assumed that as Hg is extracted into the octanol, equilibrium speciation is

rapidly reestablished in the aqueous phase until steady state concentrations of Hg in the aqueous and octanol phases are reached (Faust 1992; Mason et al. 1996a; Benoit et al. 1999a; Jay et al. 2000; Benoit et al. 2001a). This is a necessary condition for the application of Equation 3. The Hg rate constant for water exchange, a parameter that has been linked to the ligand exchange rate of metals, is one of the highest reported for trace metals ($1x\ 10^{-9}\ s^{-1}$) which supports the rapid reestablishment of equilibrium in the aqueous phase during the octanol-water extraction (Stumm and Morgan 1996). In some studies examining the octanol-water partitioning of other trace metals, it has been assumed that the kinetics are too slow for equilibrium to establish once some of the metal complexes have been extracted into octanol (Verhaar et al. 1995; Turner and Mawji 2004; Turner and Mawji 2005). In this case differences in the magnitude of the K_{ow}'s and the octanol/water volume ratio need to be considered in order to calculate the K_{ow} for different metal species (Verhaar et al. 1995; Turner and Mawji 2004a; Turner and Williamson). In other words, Equation 3 can not be used to determine the K_{ow} for individual metal species if equilibrium does not reestablish rapidly during extraction resulting in the need for a more complex equation. Data is presented below for Hg supporting the rapid reestablishment of equilibrium speciation during the extraction procedure.

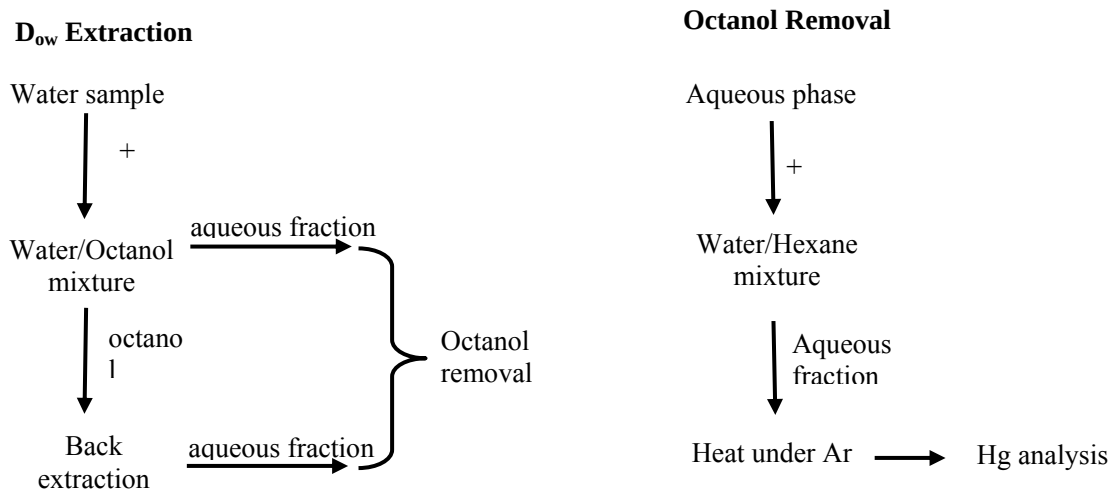


Figure 3.1: Method schematic

3.3. Method development

Previously used D_{ow} extraction techniques were modified in order to reduce the Hg concentration required to use this method, as well as to avoid analytical interferences in the Hg analysis resulting from the slight solubility of octanol in water (Figure 3.1). In order to validate this method, extractions were performed on unamended natural water samples, natural samples amended with a stable isotope of Hg, and laboratory prepared solutions containing a stable isotope of Hg. A three step cleaning procedure was used in which the labware was sequentially soaked in 1 M KOH, 25% HNO_3 and 10% HCl. The KOH cleaning removes organic matter that is not otherwise broken down by the acid cleaning process. Extractions were preformed in 120 mL FEP Teflon separatory funnels. In laboratory prepared solutions, the pH was controlled with a 0.04 M phosphate buffer prepared using KH_2PO_4 and K_2HPO_4. No buffer was added to natural water samples. For all extractions, 80 mL of the sample and 20 mL of octanol (EM Science octyl alcohol) were added to the

separatory funnels and shaken on an orbital rotation table for two hours, unless otherwise noted. After this two-hour extraction period, the aqueous and octanol phases were allowed to separate and the aqueous phase was collected into a second separatory funnel. It should be noted, that in some samples an emulsion formed between the octanol and aqueous layer. When this occurred, as much of the emulsion as possible was included in the aqueous layer but the difficulties associated with separating the layers likely resulted in some of the variability observed in the measured D_{ow}'s. For experiments involving sulfide, or for extraction of anaerobic natural waters, extractions were conducted in an anaerobic chamber and all Teflon-ware, including separatory funnels, were placed in the anaerobic chamber for a minimum of 5 days prior to use in order to remove oxygen.

Since octanol has a slight solubility in water (0.58 g/L) and octanol interferes with the Hg analysis via the $SnCl_2$ reduction/gold-trap method (Bloom and Fitzgerald 1988), traces of octanol remaining in the aqueous phase were removed using a hexane extraction. Twenty milliliters of a 4.4 M potassium chloride solution (Baker analyzed A.C.S. grade) in 5% hydrochloric acid was added to each aqueous phase sample prior to hexane extraction to ensure that >95% of the Hg was present as charged Hg-chloride complexes ($HgCl_3^-$, $HgCl_4^{2-}$) (Mason et al. 1996a) that would remain in the aqueous phase. This was followed by the addition of 15 mL of hexane (JT Baker ultra resi-analyzed 95 % n-hexane). The mixture was shaken for 1 hour. The aqueous phase was then separated from the hexane phase. Analytical interferences associated with residual hexane were avoided by purging the sample with ultra-high

purity grade argon in a 70-80^{o}C water bath for one hour to evaporate any remaining hexane.

In some previous studies (Reinfelder and Chang 1999), octanol was presaturated with water to avoid volume changes in the aqueous phase during the extraction as a result of the solubility of water in octanol. At the water/octanol water ratio used in this study, less than 1% of the water in the aqueous phase would partition into the octanol, and therefore, the octanol was not presaturated with water. Loss of water into the hexane was not a concern because the mole fraction of water in water saturated hexane in very low (0.001) (Schwarzenbach et al. 1993).

In some previous experiments (Mason et al. 1996a; Benoit et al. 1999a; Benoit et al. 2001a), the Hg concentration in the octanol phase was determined by difference, based on the Hg concentration in the sample before extraction and the amount measured in the aqueous phase after extraction. In this study, to ensure that there was no loss of Hg to Teflon-ware or evasion during the extractions, the Hg concentration in the octanol phase was also measured. A back extraction technique was developed to remove Hg from the octanol phase into an aqueous medium, allowing standard Hg analysis. This was accomplished by adding 25 mL of a 1.32 M potassium chloride solution in 4% HCl to the octanol phase after the aqueous phase had been removed. This resultant conversion of >95% of the Hg to charged Hg-chloride complexes ensures their partitioning into the aqueous solution. This aqueous solution was separated from the octanol and the residual octanol in the aqueous solution was removed as described above.

Total Hg analysis was done using standard Hg analytical methods (Gill and Fitzgerald 1987; Bloom and Fitzgerald 1988). To oxidize any organic matter, bromine monochloride was added to the samples for a minimum of twenty-four hours prior to analysis at concentrations large enough to ensure excess oxidizing potential, as indicated by residual orange color. Hydroxylamine hydrochloride was added to the samples to reduce any excess bromine monochloride. The Hg in the samples was reduced using stannous chloride immediately prior to purging and amalgamation onto traps containing gold beads. The traps were subsequentially heated and the pulse of Hg released was detected using a Hewlett Packard 4500 inductivelycoupled plasma-mass spectrometer (Hintelmann and Evans 1997b; Hintelmann and Ogrinc 2003). Hg-202 was used to determine the background concentration of Hg in all samples and Hg-199 was used as an isotope spike in amended samples. The isotope spike was added as a Hg^{199}-chloride complex. This isotope, purchased from Oak Ridge National Laboratories, is 91.95% pure. The calculations required to determine concentration of Hg from the added isotope in the samples is presented elsewhere (Hintelmann and Ogrinc 2003). A solid state ion selective electrode and a reference electrode (Thermo Electron Corporation) was used to analyze for sulfide (Eaton 1998). Sample and standards were preserved in sulfide antioxidant buffer (SAOB) and analyzed within 4 hours.

Speciation calculations were performed using the program MINEQL+ (Environmental Research Software, Hallowell, ME, USA). The formation constants for Hg-sulfide complexes (Benoit et al. 1999a) and a complexation constant for Hg to DOM based on the molar concentration of DOM, as determined by Benoit et al. 2001

(Benoit et al. 2001a), were added into the MINEQL+ database. All relevant constants are presented in Table 1.1.

3.4. Method validation

One of the objectives of modifying previously used D_{ow} extractions was to reduce Hg contamination in order to apply this method to natural water samples with low levels of Hg. The three-step bottle washing procedure, as described above, was used to reduce Hg contamination associated with the labware. Potential Hg contamination could also arise from the octanol, hexane and potassium chloride solutions used during the extractions. In order to determine the background levels of Hg associated with the labware and reagents, the revised D_{ow} extraction procedure was carried out using unamended Milli-Q water (18.2 MΩ* cm, Millipore Corp.). Several experiments were conducted to examine both the level of Hg contamination and the error associated with the blank measurement. The average Hg blank associated with the extraction of 80 mL of Milli-Q water was 33.5 ± 10.2 pg, based on three trials, each consisting of three blank measurements. The relative standard deviations were lower within each individual trial. In all subsequent extractions of natural waters, blanks were subtracted from the sample results. The detection limit calculated as three times the standard deviation of the blanks for this method is 0.4 ng/L, making this method applicable to examine Hg in unamended samples. Typical dissolved phase Hg concentrations are well below 20 ng/L in uncontaminated environments (EPA 1997). As a comparison, the concentration of Hg used in

previous octanol-water studies ranged from 0.5-200 µg/L (Mason et al. 1996a; Benoit et al. 1999a; Jay et al. 2000).

In order to examine whether Hg was lost during the extraction procedure, the mass recovery of Hg added to Milli-Q, lake and estuarine water samples was assessed at the end of the D_{ow} procedure. The use of a stable isotope for recovery studies allowed clear distinction of any Hg contamination from the spike addition. For these studies, Hg-199 was added to the water samples 12 hours prior to the extractions, at a level of 1.0 ng/L. The Hg-199 was measured in samples prior to extraction, and in the aqueous and octanol phases after extraction. Lake water was collected from Lake Lariat, a small lake in Calvert county Maryland, USA which is characterized by relatively high DOM. Estuarine river water, which contained lower DOM levels and higher chloride concentrations, was collected from the Patuxent River in Solomons, Maryland, USA. The average recovery of the added Hg-199 from the Milli-Q, lake and river water was 94.2 ± 4.2, 94.9 ± 5.7, and 95.5 ± 0.6% respectively. This recovery includes Hg in the aqueous and octanol phase, which could also include Hg adsorbed to the separatory funnel wall during the initial extraction step. Since the recovery was high and consistent across different water matrices, subsequent extractions only examined the amount of Hg in the unextracted water and the aqueous phase. The amount of Hg associated with the octanol phase was determined by the difference between the whole water and aqueous phase Hg.

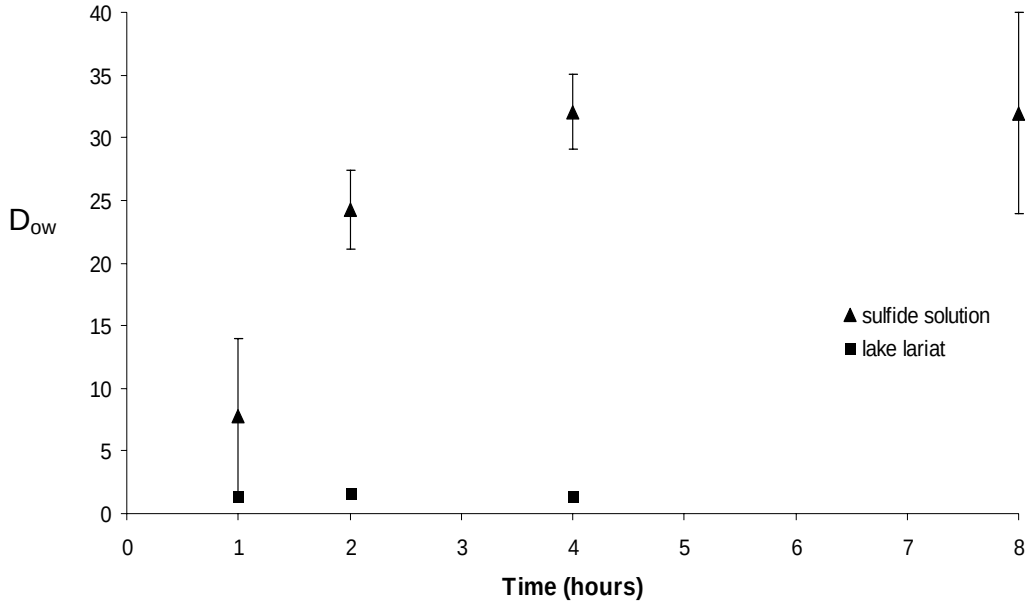


Figure 3.2: Measured D_{ow}'s over time in a sulfide solution and lake water spiked with Hg-199. The error bars represent the standard deviation of triplicate samples.

Previously used Hg D_{ow} extraction methods have employed a range of extraction times from 20 minutes to 2 hours (Major et al. 1991; Benoit et al. 1999a; Jay et al. 2000) and the extraction times used to analyze other metals have lasted as long as 2 weeks (Zimmermann et al. 2003). In order to determine the amount of time required for steady-state concentrations to establish in the aqueous and octanol phase, two extraction time series were conducted. For both experiments, samples were amended with 1.0 ng/L Hg-199. In the first time series experiment, Lake Lariat water was used and the D_{ow} was measured at 1, 2 and 4 hours. In the second experiment, a $3.2x10^{-5}$ M laboratory prepared solution of sulfide was used and the D_{ow} was measured at 1, 2, 4 and 8 hours (Figure 3.2). All laboratory solutions were prepared in a 0.01 M pH 6 phosphate buffer, unless otherwise noted, in order to maintain an

environmentally relevant pH. The used of a phosphate buffer also enabled a comparison to be drawn with previous Hg octanol-water partitioning studies in which phosphate buffers were also used to prepare laboratory solutions (Benoit et al. 1999a; Benoit et al. 2001a). Thermodynamic calculations indicate that Hg-DOM complexes are the dominant Hg complex in Lake Lariat water and there was no difference in the measured D_{ow} over time (Figure 3.2) suggesting that equilibrium is rapidly reestablished once Hg-DOM complexes are extracted into octanol. In the sulfide solution, the Hg speciation was composed of HgS^0 (8.4%), $Hg(HS)_2^0$ (33.6 %) and HgS_2H^- (57.5%). The measured D_{ow} at 2, 4 and 8 hours were not statistically different ($p= 0.01$) (Figure 3.2), supporting the idea that equilibrium is rapidly reestablished once Hg is extracted into the octanol phase. For all subsequent extractions, an extraction time of 2 hours was employed.

In order to compare D_{ow} values measured using the modified extraction procedure presented here with previously conducted extractions, the D_{ow}'s of Hg-chloride and Hg-DOM solutions were examined. An extraction was conducted on a 4.3 ng/L Hg-199 solution in 0.014 M sodium chloride at pH 6.8. Under these conditions, thermodynamic calculations predict that $HgCl_2$ (81.5%), $HgCl_3^+$ (11.4%) and HgClOH (6.4%) dominate the speciation. Using the reported K_{ow}'s for these species (Mason et al. 1996a), the fraction of the three species present in the solution and Equation 3, the predicted D_{ow} for this solution would be 3.1. The D_{ow} measured using the modified procedure, at low levels of Hg, was 3.1 ± 2.3, indicating that the modified D_{ow} extraction procedure is comparable to previously used extraction techniques.

The modified extraction technique was also applied to two sulfidic, oxygen-free Milli-Q water solutions to test the method under anoxic conditions, and to compare results with previous measurements of the K_{ow} of HgS^0 and $Hg(SH)_2^0$ (Benoit et al. 1999a). It should be noted that it is likely that HgS^0 is a hydrated complex which can be expressed as $HgSHOH^0$ (Tossell 2001). The measured D_{ow}'s were 54 ± 9 for the first solution (1.0 ng/L Hg-199, 20 μM sulfide, pH 6.1) and 28 ± 6 for the second solution (0.8 ng/L Hg-199, 30 μM sulfide, pH 6.3). Using the predicted speciation of Hg in the two solutions and employing the assumption that the K_{ow} for HgS^0 and $Hg(SH)_2^0$ are the same, the K_{ow} , calculated using Equation 3, was estimated as 72 ± 15. In calculating the K_{ow}'s of HgS^0 and $Hg(SH)_2^0$, it was assumed that charged Hg-sulfides do not partition into octanol and HgS^0 and $Hg(SH)_2^0$ partition into octanol to the same extent as shown by Benoit et al. 1999.

The calculated K_{ow} for both HgS^0 and $Hg(SH)_2^0$ were larger than the K_{ow}'s for the same species determined in Benoit et al. 1999 (K_{ow} = 25), but similar to that calculated based on Jay et al. 2000 . Using D_{ow} data for Hg-sulfide solutions presented in Jay et. al., 2000, the calculated K_{ow} for HgS^0 and $Hg(SH)_2^0$ would be 66 ± 20, a value that is similar to the K_{ow} calculated in this study. The lower value reported by Benoit et al. 1999 can likely be explained by the differences in the extraction techniques. In Benoit et al. 1999, 96% of the Hg adsorbed to the extraction container walls or precipitated during the extraction. In our study, both MINEQL+ calculations and filtration of the aqueous phase demonstrated that precipitation was insignificant and adsorption to the container walls was on average 24.6%, with the highest adsorption to the walls occurring in samples containing DOM isolates. Also,

it is possible that in the previous studies steady state concentrations were not established between the octanol and aqueous phase during the two hour extraction period as a result of the high Hg concentrations used in these studies.

Extractions were also preformed on Hg-DOM solutions, using Suwannee River natural organic matter (DOM) (reference material from the International Humic Substances Society) and a hydrophilic acid DOM isolate from the Florida Everglades (obtained from G. Aiken, USGS Boulder, CO) (Ravichandran et al. 1998). The Suwannee River DOM solution contained 30 mg/L DOM and 1.4 ng/L Hg-199 while the Florida Everglades solution contained 10 mg/L DOM and 0.6 ng/L Hg-199. Both solutions were prepared in 0.04 M, pH 6 phosphate buffer and as a result of no other strong complexing ligands in solution, the Hg was completely complexed to the DOM. The D_{ow} values for Hg bound to Suwannee River DOM and the Everglades DOM were 1.7 ± 1.1 and 3.3 ± 2.0 respectively. The large variability associated with these measurements is likely a combination of the low concentration of Hg and the difficulties associated with the separation of the aqueous and octanol phase due to the formation of an emulsion at the interface in some instances. In previous studies, samples were not collected at the interface of the aqueous and octanol phases since only small subsamples of these phases were needed for analysis. As a result of the low Hg concentrations used in this study, the entire aqueous and octanol phase were needed for analysis. Collecting the entire phase could lead to the observed variability in the measurements because the octanol-water interface was not always clearly defined.

Benoit et al. (2001) reported a D_{ow} of 0.12 ± 0.03 for the Everglades DOM used in this study, which is much lower than our measured values. Namjesmik-Dejanovic and Cabaniss (2004) used reverse-phase HPLC to estimate D_{ow}'s for a suite of DOM samples. Calculated D_{ow}'s ranged from 12.9 to 37.2 for the DOM isolates suggesting that some fraction of DOM will partition into octanol. These D_{ow}'s are much larger than our measurements D_{ow}'s for Hg-DOM complexes suggesting that only a small portion of the Hg would have to complex with the more hydrophobic portion of the DOM to explain our measurements. This is supported by the observation that a significant fraction of dissolved Hg is associated with large organic complexes or colloidal matter (Babiarz et al. 2001; Choe et al. 2003) which is more hydrophobic than Hg associated with small organic molecules. Using C_{18} columns, it has been shown that in several estuarine water samples greater than 50% of the mercury is present as hydrophobic organic complexes (Turner et al. 2001), also supporting the D_{ow}'s measured for Hg-DOM complexes in this study. Differences in the D_{ow} values presented here compared to Benoit et al., 2001, could also be a result of differences in the extraction techniques, as discussed above.

3.5. Natural sample

Extractions were preformed on surface water collected from Lake Lariat and interstitial water separated from sediments collected at Lake 658 at the Experimental Lakes Area, Ontario, Canada, a lake that has been amended with Hg-202 since 2001. Sediment was collected from Lake 658 in July 2004 and held under anaerobic

conditions for 48 hours before the interstitial water was isolated. Nalgene 0.2 μM disposable filter units were used to separate the interstitial water from the sediment matrix in an anaerobic chamber. The ambient Hg concentrations for Lake Lariat and Lake 658 interstitial water were 0.8 ng/L and 1.2 ng/L, respectively. The surface water from Lake Lariat was oxic and thermodynamic calculations indicated that the Hg would be bound to organic complexes. The sulfide concentration in the interstitial water collected from Lake 658 was below the detection limits of our sulfide analysis ($<1 \times 10^{-7}$ M) and therefore the Hg complexation was predicted to be controlled by Hg-DOM complexes. Both of these extractions were conducted under anaerobic conditions.

The measured D_{ow} from Lake Lariat surface water and from Lake 658 interstitial water were 1.1 ± 0.4 and 1.2 ± 0.2 respectively. These are lower than the D_{ow} determined for DOM isolates, which ranged from 1.7 to 3.3 but it is not unreasonable to assume that the Hg in these natural water samples are bound to DOM since it has been shown that the polarity of DOM varies between systems (Namjesnik-Dejanovic and Cabaniss 2004). It is not possible with this technique to eliminate the presence of inorganic Hg complexes, such as Hg-chloride complexes, since the K_{ow} for Hg-DOM and $HgCl_2$ are similar. As a result of the low chloride concentration and the high affinity of Hg to organic matter, MINEQL+ calculations support the notion that it is unlikely that any inorganic Hg complexes are present in this matrix.

3.6. Application of method

The modification of previously used octanol-water partitioning techniques enables this method to be used to examine the D_{ow} of Hg complexes in natural samples without the addition of high concentration or radioactive Hg. In previous studies using this technique Hg concentrations of 500 ng/L or greater have been used, while the modification to this method presented here decrease the detection limit to 0.4 ng/L. The D_{ow}'s measured for Hg-DOM complexes present here are higher than previously reported values suggesting that these complexes are more hydrophobic than previously believed. Unfortunately the D_{ow}'s for Hg-DOM complexes overlap the K_{ow} for $HgCl_2$ making it impossible to determine if the dominate Hg complex in oxic water is a chloride or DOM complex, although comparisons of results in conjunction with speciation calculations allows a conclusion to be reached. However, if octanol-water extractions are used in conjunction with thermodynamic equilibrium calculations, they can be a powerful tool to demonstrate that the equilibrium estimations are valid, and to probe the possibility of other interactions that have not been previously accounted for, as shown here in the later chapters.

While this technique can not differentiate between organic and inorganic Hg complexes in oxic water under some conditions, it can be extremely useful in examining the speciation of Hg in the presence of sulfide. The K_{ow} of neutral Hg-sulfide species, such as HgS^0 and $Hg(SH)_2^0$(K_{ow} = 72), are much larger than the K_{ow}'s of other Hg complexes, and therefore, octanol-water extractions are the ideal tool to investigate the concentrations of these key Hg species in natural waters. The data presented here, coupled with previous laboratory studies (Benoit et al. 1999a; Jay et

al. 2000) shows that this technique can be applied to sulfidic, low level Hg samples. The presence of neutral Hg-sulfide complexes has been positively correlated with the bacterial methylation of Hg as a result of the passive uptake and subsequent methylation of these complexes by bacteria. The importance of neutral Hg-sulfide complexes has been demonstrated using laboratory experiment, but these species have not been measured nor their presence detected in the natural samples. Thermodynamic models, which have not be validated with actual complex sample matrixes which exist in natural samples, are the only means of estimating the abundance of neutral Hg-complexes in the environment.

The modifications to the octanol-water partitioning extraction procedure eliminates contamination and analytical interferences but at the same time the relative standard deviations associated with the measured D_{ow} increased. Even though the relative standard deviations are large for some of the samples presented here, this will not affect the differentiation between neutral Hg-sulfide complexes and other Hg complexes because of the much larger K_{ow} for the neutral Hg-sulfide complexes.

Chapter 4: The influence of dissolved organic matter on the complexation of mercury under sulfidic conditions

4.1. Introduction

The concentration of methylmercury (MeHg), the most toxic and bioaccumulative form of mercury in aquatic organisms, is dependent on the net production of MeHg in aquatic systems, with biotic production being more important than abiotic process (St Louis et al. 1996; Driscoll et al. 1998). The complexation of inorganic mercury (Hg) is a controlling factor in the bacterial production of MeHg (Barkay et al. 1997; Benoit et al. 1999b; Benoit et al. 2001b; Benoit et al. 2001c). The speciation of Hg under oxic conditions has been demonstrated to be dominated by organic Hg complexes in most environments (Hintelmann et al. 1997a; Babiarz et al. 2001; Benoit et al. 2001a; Choe et al. 2003; Hsu and Sedlak 2003; Lamborg et al. 2003; Ravichandran 2004), with the exception of high salinity areas in which chloride complexes are also important (Mason et al. 1996a). Thermodynamic models suggest that inorganic Hg-sulfide complexes dominate the speciation of Hg under sulfidic conditions (Figure 1.2) (Dyrssen and Wedborg 1991; Benoit et al. 2001a).

Biotic production of MeHg is mediated by sulfate reducing bacteria (SRB) (Compeau and Bartha 1985; Gilmour et al. 1992; Gilmour and Riedel 1995) and the concentration of neutral Hg-sulfide complexes has been shown as a controlling factor in the microbial production of MeHg by SRB's in both aquatic systems (Benoit et al.

1998; Gilmour et al. 1998; Benoit et al. 1999b) and laboratory bacterial cultures (Benoit et al. 1999a; Benoit et al. 2001b; Benoit et al. 2001c). It has been demonstrated that the rate of Hg methylation by *Desulfobulbus propionicus*, a SRB, is dependant on the concentration of neutrally charged Hg-sulfide complexes, which appear to be taken up via passive diffusion (Benoit et al. 1999a; Benoit et al. 2001b; Benoit et al. 2001c). Microbial "bioreporters" have also been used to assess the bioavailability of Hg for uptake by microorganisms. While such organisms offer insights into Hg transport pathways, they provide no direct measurement of Hg complexation or methylation. Bioreporters studies using an *E. coli*-based *mer-lux* bioreporter also showed that neutrally-charged Hg species were more readily taken up than charged species, and that complexation with higher molecular weight organic matter strongly inhibited Hg uptake (Barkay et al. 1997). However, work with *Vibrio anguilara* and *E. coli*-based bioreporter strains showed enhancement of Hg uptake in the presence of low molecular weight organic acids, suggesting facilitated transport mechanisms for charged Hg complexes (Golding et al. 2002; Kelly et al. 2003). It is evident that Hg complexation is extremely important in Hg cycling, but our current knowledge often relies on thermodynamic speciation models to predict Hg speciation, since the complexation of Hg under sulfidic conditions has not been examined outside of the laboratory.

As a result of SRB mediated Hg methylation, it has been found that the active zone of Hg methylation in ecosystems corresponds with the active zone of sulfate reduction, which usually occurs in the sediment (Benoit et al. 2003). The reduction of sulfate to sulfide in these zones leads to the accumulation of sulfide in the

interstitial water and as a result of the strong affinity of Hg to sulfide, the speciation of Hg should be controlled by Hg-sulfide species. Sulfide concentration and pH control the distribution of Hg-sulfide complexes. While the abundance and composition of Hg-sulfide complexes have never been measured in natural systems, thermodynamic models, using complexation constants which have been experimentally determined or extrapolated from other metal sulfide complexes (Benoit et al. 1999b), have been used to predict the speciation of Hg under sulfidic conditions. Under most natural conditions the dominate Hg-sulfide complexes are HgS^0, $Hg(SH)_2^0$, $HgHS_2^{1-}$ and HgS_2^{2-} (Figure 1.2b) (Benoit et al. 2003) but there is also the possibility of Hg complexation with polysulfides under some conditions (Jay et al. 2000).

Only the neutrally charged Hg-sulfide complexes (HgS^0 and $Hg(SH)_2^0$) appear available for uptake and subsequent Hg methylation by sulfate reducing bacteria (Benoit et al. 1999a; Benoit et al. 2001c). The diffusion rates of HgS^0, calculated using octanol-water partitioning experiments, exceeds measured methylation rate in laboratory studies, suggesting that either all the Hg which enters the cell in not methylated or that the calculated diffusion rate of Hg into the cell is overestimated. Using quantum mechanical calculation, it has been shown that HgS^0 is not stable as an aqueous complex and would actually exist of $Hg(SH)OH^0$ (Tossell 2001). As a result of the larger molar volume, calculated diffusion rates for $Hg(SH)OH^0$ are smaller relative to HgS^0, but the estimated diffusion rate still provides Hg inside cells in excess of what is needed to support observed methylation rates (Benoit et al. 2003). Recently reported octanol-water partitioning coefficients for neutral Hg-sulfide

complexes are larger than the values used to calculate the diffusive uptake of Hg into the cell (Chapter 3). Using these higher partitioning coefficients to calculate diffusion rates, would increase the estimated amount of Hg entering the cell leading to an even larger discrepancy between estimates of Hg entering the cell and rates of methylation. One of several possible explanations for this discrepancy could be the over estimation of the abundance of neutral Hg-sulfide complexes in natural water.

Since the speciation of Hg has never been measured under sulfidic conditions, thermodynamic speciation models used to predict the complexation of Hg in the presence of sulfide, have not been validated. Furthermore, the finding that organic matter potentially influences the complexation of Hg in the presence of sulfide in waste water samples indicates a broader evaluation of Hg speciation under anoxic conditions is required (Hsu-Kim and Sedlak 2005). Using competitive ligand exchange and C-18 solid phase extraction, Hsu-Kim and Sedlak (2005) showed that in the presence of glutathione, Hg-sulfide complexes were retained on C-18 columns, while Hg-humic acid complexes were eluted from the column. In a solution containing both humic acid and sulfide, Hg was only partially retained on the C-18 column when glutathione was added. The partial elution of Hg from the column was explained by either an association of Hg-sulfide complexes with the humic acid or a change in the properties of the C-18 column as a result of the humic acid. Also, dissolved organic matter (DOM) has been shown to both enhance the dissolution and inhibit the precipitation of solid $HgS_{(s)}$ (Ravichandran et al. 1998; Ravichandran et al. 1999; Waples et al. 2005), indicating that organic matter has the potential to interact with Hg even in the presence of sulfide.

While laboratory studies have shown the importance of neutral Hg-sulfide complexes in controlling the rate of Hg methylation, the predicted abundance of these species has never been verified in natural water samples. Octanol-water partitioning provides a means to measure neutral Hg-sulfide species in natural systems as a result of the greater tendency of these species to partition into octanol relative to other Hg species (Benoit et al. 1999a; Chapter 3). Recently, we have shown that improvements to previously used octanol-water partitioning techniques have lowered the method detection limit of for Hg, so that it can be applied to unamended natural samples (Chapter 3). Ultrafiltration provides another means to examine the complexation of Hg under sulfidic conditions. Cross flow ultrafiltration has been used to examine the distribution of Hg between different size classes of organic matter (Babiarz et al. 2000; Babiarz et al. 2001; Babiarz et al. 2003; Choe et al. 2003), but to our knowledge, it has never been used to examine Hg complexation in anoxic samples. Ultrafiltration can be used to differentiate between small inorganic complexes and larger organic complexes of Hg.

The modified octanol-water partitioning (D_{ow}) method presented in Chapter 3 was used to determine the abundance of neutral Hg-sulfide complexes in natural interstitial water. A significant discrepancy was observed between the predicted and observed Dow for Hg in sulfidic waters containing DOM. Therefore, the interaction of DOM with Hg under sulfidic conditions was investigated in detail in laboratory prepared solutions. Using D_{ow} extractions and ultrafiltration, and interaction between NOM, Hg and sulfide was confirmed. This interaction is not currently predicted from known thermodynamic constants.

4.2. Methods

4.2.1. Octanol-water extraction method

Octanol-water partitioning extractions were preformed in 120 mL FEP Teflon separatory funnels. For all extractions 80 mL of the sample and 20 mL of octanol (EM Science octyl alcohol) were added to the separatory funnels and shaken on an orbital rotation table for two hours. The aqueous phase was collected into a second separatory funnel. Twenty milliliters of a 4.4 M potassium chloride solution (Baker analyzed A.C.S. grade) in 5% hydrochloric acid and 15 mL of hexane (JT Baker ultra resi-analyzed 95 % n-hexane) were added to the aqueous phase and shaken 1 hour in order to remove residual octanol from the solution. The separated aqueous phase was purged with ultra-high purity grade argon in a 70-80^{o}C water bath for one hour to evaporate any remaining hexane. Extractions were conducted in an oxygen-free anaerobic chamber and all Teflon-ware, including separatory funnels, were placed in the anaerobic chamber for a minimum of 5 days prior to use in order to remove oxygen. A detailed description of the octanol-water partitioning method can be found in Chapter 3.

Mercury was analyzed in the aqueous phase. Mercury concentrations in the octanol phase were calculated by difference from the total, corrected for any loss to the separatory funnels walls. Wall loss was estimated from a separate extraction without octanol. Extraction blanks were performed using Milli-Q (18.3 MΩ*cm,

Table 4.1:Known K_{ow} values

Species	K_{ow}	Species	K_{ow}
$Hg(OH)_2^0$	1.2[a]	$HgSH^+$, HgS_2^{2-} *	0
$HgCl_2^0$	3.3[a]	HgS^0, $Hg(SH)_2$	72[b]
HgOHCl	0.05[a]	HgDOM**	1.7[b]
$HgCl^+$	0*	HgDOM***	3.3[b]

* Charged complexes are assumed to not significantly partition into octanol (Benoit et al., 1999)
** K_{ow} of Hg complexed to Suwannee River NOM
*** K_{ow} of Hg complexed to DOM isolated from the Florida Everglades
a Mason et al 1996
b Chapter 3

Millipore Corp.) water and the Hg associated with these blanks was also subtracted from the samples.

The partitioning coefficient of a pure Hg species (K_{ow}) is defined using Equation 1, where HgL_{oct} and HgL_{aq} are the concentrations of Hg bound to a specific ligand in the octanol and aqueous phase, respectively. The octanol-water partitioning coefficients of several Hg complexes have previously been measured (Table 4.1) (Chapter 3; Mason et al. 1996a; Benoit et al. 1999a). When the octanol-water partitioning of Hg in a water sample is measured, an overall partitioning coefficient (D_{ow}) is determined since several Hg complexes could be present. The D_{ow} is the ratio of the total Hg concentration in the octanol phase, Hg_{oct}, to the total Hg concentration in the aqueous phase, Hg_{aq} at the end of the extraction (Equation 2). The K_{ow} of individual Hg complexes ($(K_{ow})_i$) are related to the D_{ow} of a sample by the fraction of the individual complexes (α_i) present in the sample (Equation 3) (Faust 1992).

Equation 1: $K_{ow} = HgL_{oct}/HgL_{aq}$

Equation 2: $D_{ow} = Hg_{oct}/Hg_{aq}$

Equation 3: $D_{ow} = \sum \alpha_i (K_{ow})_i$

4.2.2. D_{ow} of mercury in estuarine sediment interstitial water

The concentration of neutral Hg complexes was examined in estuarine interstitial waters using octanol-water partitioning in order to determine if thermodynamic model correctly predicted the speciation of Hg under sulfidic conditions. Sediments were collected at Kingfisher Pond, a small cove off of the Patuxent River, in California, MD. PVC core barrels, with a 4.8 cm diameter, were used to collect sediment to an approximate depth of 10 cm. The interstitial water was separated from the sediment in an anaerobic chamber by vacuum filtration (0.45 μM) using disposable polycarbonate Nalgene filter units with cellulose nitrate filters. Triplicate octanol-water extractions were performed on the separated interstitial water. The sulfide concentration and pH of the interstitial water was also determined. Sulfide was analyzed within 4 hours of collection, using an ion-specific electrode on samples preserved in sulfide-antioxidant buffer (Eaton 1998).

4.2.3. Dow of mercury in an Estuarine Sediment Slurry

In order to examine the speciation of Hg in a more controlled system, while still preserving the important interactions occurring under natural conditions, a sediment slurry was prepared in the laboratory using 200 g of surficial (0-10 cm) estuarine sediment, collected from Mackall Cove, a small inlet off the Patuxent River in St. Leonard, MD, and 2 L of filtered deoxygenated Patuxent River water. A stable

isotope of Hg was added to the sediment slurry so that background Hg contamination would not be a concern for this experiment. An enriched Hg-199 (91.95% purity) stable isotope was used to obtain approximately 125 ng/L Hg-199 in the slurry. The enriched stable mercury isotope was obtained from Oak Ridge National Laboratories and dissolved in a dilute acid solution. For all experiments, the Hg-199 was added to solutions as a Hg^{199}-chloride complex. The slurry was shaken in an anaerobic chamber for 2 days to allow for the bacteria production of sulfide and the partitioning of the isotope spike between the solid and dissolved phases. Prior to the D_{ow} extraction, the water in the slurry was separated from the solid phase using quartz fiber filters (QMA). Octanol-water extractions were performed in quadruplicate on the isolated water. The sulfide concentration and pH of the slurry water was also determined.

4.2.4. Dow of mercury/sulfide/DOM mixtures

To examine the influence of DOM on the speciation of Hg in the presence of sulfide, octanol-water extractions were performed on laboratory solutions of Hg with two DOM isolates, each with and without sulfide. Suwannee River natural organic matter, was purchased from the International Humics Substance Society, and a hydrophilic isolate from the Florida Everglades (2BS-HPiA) was obtained from G. Aiken (USGS Boulder, CO). The Suwannee River DOM is a freeze dried isolate collected using reverse osmosis resulting in a sample containing hydrophobic humic and fulvic acids, along with other soluble organics. The Florida Everglades DOM isolate was collected in the northern Everglades within the Water Conservation Area

Table 4.2: Characteristics of DOM isolates from the Suwannee River and the Florida Everglades used in the laboratory prepared solutions.

DOM	Isolation method	Average molecular weight	%C	%S	Reduced S (% of total S)	Carboxyl (mol kg^{-1} C)	Aromatic C (NMR) (%)
[1] Suwannee River NOM	Reverse Osmosis	nr	48.8	0.6	nr	9.85	nr
[2]F1-HPoA	XAD resin	1031	52.2	1.73	22.1	10.43	18.2

nr not reported
[1] Data for the Suwannee River NOM was obtained from the International Humic Substances Society
[2] F1-HPoA is a hydrophilic acid isolated from within Water Conservation Area 2A of the Florida Everglades and was provided by Dr. George Aiken, USGS Boulder, CO. Characteristics of this isolate were reported in Ravichandran (1999)

2B and details of the location and characteristics of this isolate are presented elsewhere (Ravichandran et al. 1999). The characteristics of the two DOM isolates are shown in Table 4.2. The DOM isolates were dissolved in a 0.04 M, pH 6 deoxygenated, phosphate buffer in an anaerobic chamber to a final concentration of 15 mg C/L. A phosphate buffer was used for all solutions in this study to maintain a pH in the solutions that was environmentally relevant. After the DOM solution was allowed to equilibrate for a couple of hours a saturated solution of sodium sulfide was added to half of each solution to reach a sulfide concentration of approximately 20-30 μM sulfide. Hg-199 was also added to reach an approximate concentration of 1.25 ng/L Hg-199 and the solutions were allowed to equilibrate overnight before the extraction. Extractions were performed as describe above for slurry experiment.

4.2.5. Ultrafiltration separation method

A centrifugal ultrafiltration method was developed as another approach to examining the interactions of mercury, DOM and sulfide. Millipore Amicon Ultra-15 centrifugal filters with a nominal molecular weight limit of 5000 Da were used to separate Hg associated with organic matter from low molecular weight Hg complexes. Prior to use, the filters were rinsed five times with Milli-Q water to ensure the removal of any organic complexes used in filter production. For all experiments, 15 mL of solution was aliquoted into the filters and centrifuged at 4000 rpm on a Sorvall RT 6000 swinging bucket centrifuge The Hg concentration of the solutions passing through the filter (filtrate) and remaining above the filter were analyzed. The spin time used with this molecular weight cut off filter ensured that greater than 90% of the complexes greater than 5000 Da remained above the filter (Millipore 2003). For all experiments using the centrifugal ultrafilters, a stable isotope of Hg was used and as a result, Hg contamination associated with the filters was not a significant concern.

4.2.6. Method Validation

We believe that this is the first study using centrifuge ultrafiltration to examine Hg complexation, and, therefore, in order to demonstrate the applicability of the ultrafilters for this purpose, several solutions were prepared to examine the distribution of Hg remaining above the filter and in the filtrate. Solutions were prepared in pH 6, 0.04 M phosphate buffer for all extractions. Five solutions were used, each containing only one Hg complexing ligand, to test the utility of the

Table 4.3: Ultrafiltration validation experimental results

Solution	Speciation	recovery	% above filter	% in filtrate	% on filter
No ligands	$Hg(OH)_2$ 100 %	90%	11%	21%	68%
0.01 M Chloride	$HgCl_2$ 91.3% $HgCl_3^-$ 7.3% HgClOH 1.1%	93%	11%	21%	68%
0.01 M acetate	$Hg(acetate)_4^{2-}$ 98.2 % $Hg(acetate)_3^-$ 1.7 %	96%	8%	16%	75%
1.9 x 10^{-5}M sulfide	HgS^0 53.1% HgS_2H^- 37.3 %	99%	16%	14%	70%
12.1 mg C/L DOM	Hg-DOM	102%	86%	2%	12%

ultrafilters for examining the association of Hg with different Hg-complexes. One solution was prepared without the addition of any strong Hg complexing ligands, resulting in the dominate Hg complex being $Hg(OH)_2$. Solutions were also prepared using NaCl (0.01 M), acetate (0.01 M), sulfide (1.9 x 10^{-5} M) and DOM (12.1 mg C/L) and the dominant species in each of these solutions, determined through thermodynamic calculation, is shown in Table 4.3. The sulfide solution was prepared in an anaerobic chamber using deoxygenated pH 6 phosphate buffer to prevent the oxidation of the sulfide. Suwannee River DOM was used to prepare the DOM solution. An initial solution containing 35 mg DOM (17 mg C) was dissolved in 150 mL phosphate buffer. This solution was ultrafiltered and the DOM greater than 5000 Da was diluted in phosphate buffer and filtered through a glass fiber filter (GFF). This solution was farther diluted with phosphate buffer to obtain a 12.1 mg C/L DOM solution containing only DOM greater than 5000 Da. For all solutions, enriched Hg-

199 was added to an approximate concentration of 24 ng/L. Ultrafiltration was performed on the solutions after they had equilibrated overnight. Inorganic complexes of Hg (Hg-chloride, Hg-hydroxide and Hg-sulfide) and Hg complexed to acetate should all pass through the filter since these complexes are less than 5000 Da, while the Hg-DOM complexes should remain above the filter.

Three 15 mL aliquots of each solution were ultrafiltered. In order to prevent the sorption of Hg, associated with the filtrate, to the walls of the centrifuge tubes, 300 uL of 50% HCl was added to the bottom of the centrifuge tubes so that the filtrate was acidified to 1% HCl during filtration. After filtration, the solution above the filter was collected by rinsing the filters with three 5 mL aliquots of phosphate buffer and pouring off the sample into a separate centrifuge tube. This solution was acidified to 1% HCl. In order a examine the amount of Hg sorbing to the filters, 15 mL of a bromine monochloride solution (0.13M KBr; 0.06M $KBrO_3$) was added to the filters, after the solution remaining above the filter was collected. The bromine monochloride solution was held in the filter for a minimum of 24 hours and the amount of Hg extracted in this solution was determined. The Hg in the unfiltered solution along with the Hg in solution above the filter, filtrate and sorbing to the filter was measured and the recovery of the Hg-199 was determined.

4.2.7. Examination of Hg complexation using ultrafiltration of DOM and DOM/HS

In order to farther investigate the influence of DOM on the complexation of Hg under sulfidic conditions, ultrafiltration was used on solutions containing Hg, DOM and sulfide. DOM solutions were prepared at three different DOM

concentrations (19.2, 12.1, and 5.9 mg C/L), as described above, using Suwannee River NOM greater than 5000 Da. The DOM solutions were prepared in an anaerobic chamber using deoxygenated phosphate buffer. Each of the DOM solutions were split and sodium sulfide was added to half of the solution resulting in one DOM solution without sulfide and one with sulfide at each of the DOC concentrations. The sulfide was added to obtain an approximate concentration of 10 μM. Hg-199 was added to each solution (~24 ng/L), at the same time as the sulfide was added, and the solutions were equilibrated overnight. Each solution was ultrafiltered in triplicate. The amount of Hg in the unfiltered solution, the filtrate and the solution remaining above the filter was determined. The amount of Hg sorbing to the filter was also determined by added a BrCl solution to the filter for a minimum of 24 hours.

4.2.8. Analytical methods

Total Hg analysis was done using standard Hg analytical methods (Gill and Fitzgerald 1987; Bloom and Fitzgerald 1988) with ICP-MS detection (Hintelmann and Evans 1997b). Stable isotope analysis of Hg by ICP-MS has been used in several studies in our laboratory (Heyes et al. 2004; Kim et al. 2004; Heyes et al. in press; Kim et al. in press; Sunderland et al. in press; Whalin and Mason in press) and the detection limit for this method is 10 pg/L Hg in aqueous samples. To oxidize any organic matter, bromine monochloride was added to the samples for a minimum of twenty-four hours before analysis. Prior to analysis, hydroxylamine hydrochloride was added to the samples to reduce any excess bromine monochloride. The Hg in the

samples was reduced using stannous chloride immediately prior to purging and amalgamation onto traps containing gold beads. The traps were heated and the pulse of Hg released was detected using a Hewlett Packard 4500 inductively-coupled plasma mass spectrometer (Hintelmann et al. 1997a; Hintelmann and Ogrinc 2003). Hg-202 was used to determine the background concentration of Hg in all samples and Hg-199 was used as an isotope spike in amended samples as described above. The calculations associated with determining the amount of Hg in the samples that is a result of the isotope spike are presented elsewhere (Hintelmann and Ogrinc 2003). A solid state ion selective electrode and a reference electrode (Thermo Electron Corporation) was used to analyze for sulfide (Eaton 1998). Sample and standards were preserved in sulfide antioxidant buffer (SAOB) and analyzed within 4 hours. DOC samples were analyzed using a Shimadzu TOC-5000 analyzer by the Nutrient Analytical Services Laboratory at the Chesapeake Biological Laboratory.

4.2.9. Thermodynamic modeling

Speciation calculations were performed using the program MINEQL+ (Environmental Research Software, Hallowell, ME, USA). The formation constants for Hg-sulfide complexes (Benoit et al. 1999a) and a complexation constant for Hg to DOM based on the molar concentration of DOM, as presented in Benoit et al. 2001 (Benoit et al. 2001a), were added into the MINEQL+ database. All other complexation constants were used directly from the MINEQL+ database. The important complexation constants for Hg are listed in Table 1.1 in Chapter 1.

4.3. Results

4.3.1. Octanol-water partitioning

Measured D_{ow}'s for Hg in sulfidic estuarine interstitial waters and artificial solutions of Hg, sulfide and NOM were much lower than predicted based on thermodynamic equilibrium modeling using the constants for Hg-sulfide and Hg-DOM complexes given in Table 1.1 (Fig 4.1). Because the affinity of Hg for sulfide is much higher than for DOM (Benoit et al. 2001a; Ravichandran 2004), models for Hg complexation under sulfidic conditions predict that inorganic sulfide complexes (HgS^0, $Hg(SH)_2^0$, $HgHS_2^{-1}$, HgS_2^{2-}) will be dominant (Figure 1.2). At sulfide concentrations in the low micromolar range, the neutral complex should dominate, and D_{ow}'s should be high, reflecting the K_{ow} of ~70 for HgS^0. However, this did not appear to be the case for any of the natural or experimental samples extracted.

For example, the interstitial water collected from the Patuxent River had a Hg concentration of 2.3 ng/L, a sulfide concentration of 2.2 μM and a pH of 7.25. Using the thermodynamic constants in Table 1.1, the speciation of Hg in the interstitial waters should be dominated by HgS^0 (68%), $HgHS_2^-$ (30%) and HgS_2^{2-}(2%). The predicted D_{ow}, calculated using Equation 3, plus the predicted speciation and the K_{ow}'s for the dominant species, was 49.0 ± 10. However, the measured D_{ow} was 5.4 ± 3.0 (Figure 4.1).

Similar discrepancies between the predicted and observed D_{ow}'s were also observed with the water collected from the estuarine sediment slurry and from

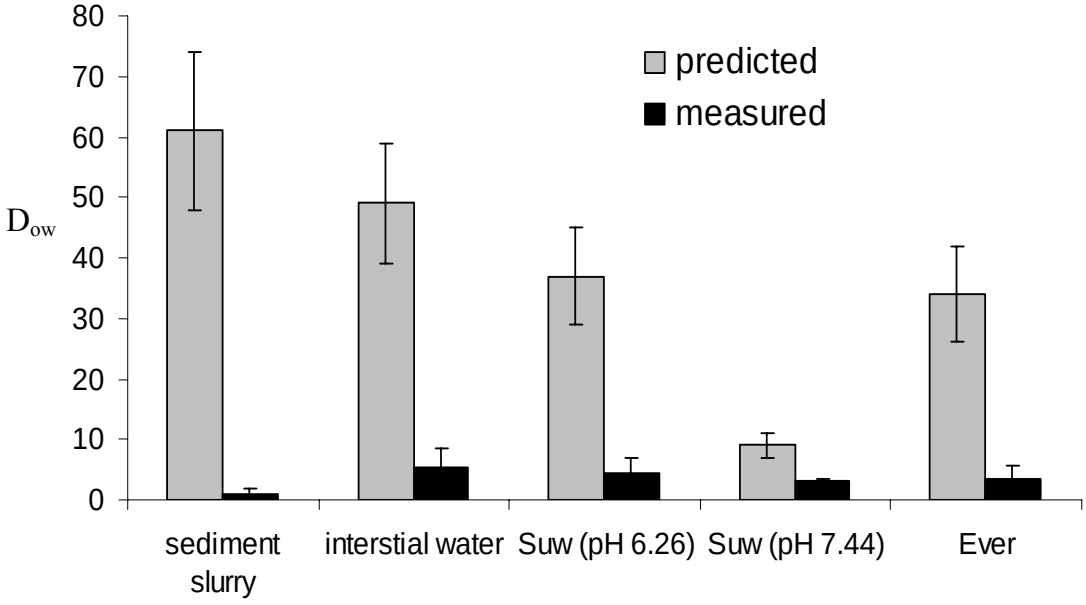


Figure 4.1: Predicted (gray bars) and measured (black bars) D_{ow}'s for the sediment slurry, interstitial water and three laboratory prepared solutions. Two of the laboratory solutions contained Suwannee River natural organic matter (Suw) at two different pH's and the third solution contained DOM from the Florida Everglades (Ever). Sulfide was added to each of these solutions. The predicted D_{ow}'s were determined from thermodynamic speciation of Hg and known K_{ow}'s for Hg-sulfide and Hg-DOM complexes

laboratory-prepared solutions of mercury, sulfide, and DOM isolates (Figure 4.1). The Hg and sulfide concentrations, and pH for each of these experiments, along with the predicted Hg speciation, are presented in Table 4.3. In all cases, the measured D_{ow} was well below the predicted value (Figure 4.1).

The low measured D_{ow}'s relative to the predicted values suggests that neutral Hg-sulfide complexes are not present or are present at much lower concentrations than predicted based on measured stability constants for HgS^0, $Hg(HS)_2^0$ and Hg-

Table 4.4: Characteristics of the natural and laboratory solutions used in the D_{ow} extractions. The speciation was calculated using the constants presented in Table 1.1

Experiment	pH	Sulfide (μM)	speciation	
Interstitial water	7.25	2.2	HgS^0	68.1%
			$Hg(SH)_2^0$	0%
			$HgHS_2^{1-}$	29.7%
			HgS_2^{2-}	1.7%
Sediment slurry	6.50	2.4	HgS^0	83.4%
			$Hg(SH)_2^0$	1.5%
			$HgHS_2^{1-}$	14.0%
			HgS_2^{2-}	0%
Suwannee DOM	6.29	20.8	HgS^0	44.1%
			$Hg(SH)_2^0$	7.8%
			$HgHS_2^{1-}$	47.6%
			HgS_2^{2-}	0%
Suwannee DOM	7.44	26.0	HgS^0	13.1%
			$Hg(SH)_2^0$	0%
			$HgHS_2^{1-}$	79.0%
			HgS_2^{2-}	6.9%
Everglades	6.25	29.7	HgS^0	37.1%
			$Hg(SH)_2^0$	9.5%
			$HgHS_2^{1-}$	52.9%
			HgS_2^{2-}	0%

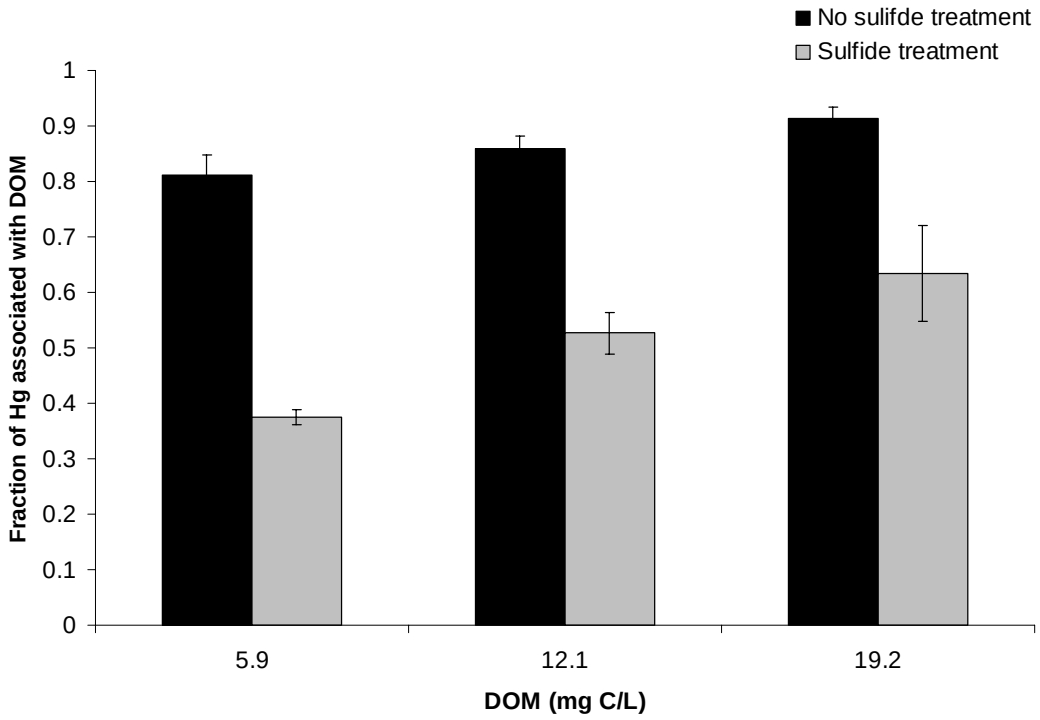
DOM alone. It is important to note that since octanol-water extractions can only detect the presence of neutral Hg-sulfide complexes, it can not be determined from this data if charged Hg-sulfide complexes are still present in the aqueous phase. It is clear however, that DOM influences the complexation of Hg under sulfidic conditions. Since the complexation constant for this interaction is not know, thermodynamic models current can not accurately predict the speciation of Hg under sulfidic conditions.

4.3.2. Ultrafiltration

The results from the validation study for the ultrafiltration method (Table 4.3) demonstrate its applicability in examining Hg complexation. For all solutions, the recovery of the added Hg isotope was greater than 90%. In solutions containing Hg complexes of less than 5000 Da (Hg-acetate, Hg-chlorides, Hg-sulfides and Hg-hydroxides) 16% or less of the Hg remained above the filter after centrifugation. A large portion of the Hg (68-75%) sorbed onto the filter (measured as Hg desorbing from the filter with the addition of bromine monochloride) while the remaining Hg was in the filtrate.

Ultrafiltration of the Hg-DOM solutions farther validated this method for use in examining the complexation of Hg. In the solution containing Hg and DOM, all of the Hg should be complexed to the DOM and, therefore, remain above the filter after filtration. The retention of Hg above the filter was 86%, while 12% and 2% was sorbed to the filter or present in the filtrate respectively. The recovery of the added Hg was essentially100 % (Table 4.2). The ultrafiltration results of solutions containing large Hg-DOM complexes and smaller organic and inorganic Hg complexes support the use of this technique to examine the association of Hg with large complexes. For all experiments using this technique, it was assumed that all of the Hg remaining above the filter was associated with DOM and, therefore, this fraction of Hg will be referred to as DOM associated Hg. It should be noted, that as a result of the slight adsorption of Hg-DOM complexes to the filters, the abundance of Hg-DOM is likely slightly underestimated using this technique.

Figure 4.2: Ultrafiltration results for solutions containing three concentrations of Suwannee River DOM in the absence (black bars) and presence of sulfide (gray bars)



The complexation of Hg was examined at three DOM concentrations in the presence and absence of micromolar levels of sulfide. When sulfide was not present, greater than 80% of the Hg was DOM associated indicating that Hg-DOM complexes dominated the Hg speciation (Figure 4.2) as would be predicted from thermodynamic models. When sulfide was present, a significant amount of Hg passed through or sorbed to the filter, but much less than ~100% that would be predicted if Hg sulfides were the predominant complexes. The amount of Hg remaining above the filter was between 38-63% indicating that a mixture of small inorganic Hg-sulfide complexes

and larger molecular weight Hg-containing DOM complexes were present. The amount of Hg not passing through the filter increased with increasing DOM concentration suggesting that the interaction between Hg, sulfide and DOM is DOM concentration dependent.

4.4. Discussion

There appears to be an interaction between mercury, sulfide and DOM that is not captured by the single ligand complexation coefficients for Hg with sulfide and DOM. In both natural sulfidic interstitial waters and artificial solutions of Hg, sulfide and DOM, the measured D_{ow} were lower than would be predicted if the speciation of Hg was dominated by inorganic Hg-sulfide complexes. This interaction is farther supported by ultrafiltration experiments, which indicate that Hg is associated with DOM in the presence of sulfide. As a result of the much stronger interaction of Hg with inorganic sulfide relative to DOM, thermodynamic equations and calculations do not accurately predict the interaction which is occurring between Hg, sulfide and DOM.

Several studies have shown that DOM inhibits the formation and enhances the dissolution of solid $HgS_{(s)}$ minerals (Ravichandran et al. 1998; Ravichandran et al. 1999; Waples et al. 2005), an interaction which could be similar to the interaction between DOM and Hg-sulfide complexes observed in our study. In those studies, correlations were found between the extent of dissolution of $HgS_{(s)}$ minerals and the molecular weight, aromaticity and specific ultraviolet absorbance of different DOM isolates. It has been proposed by those authors that DOM could be interacting with

the surface of the solid $HgS_{(s)}$ resulting in the dissolution of Hg from the solid as a dissolved Hg-DOM complex. However, as shown in Chapter 2, DOM did not influence the binding of Hg to AVS suggesting that it is not likely that a dissolved Hg-DOM complex is present. The most likely interaction involved in the increased dissolution of $HgS_{(s)}$ in the presence of DOM, is through the formation of dissolved Hg-S-DOM complexes and the presence of these complexes is increasing the overall solubility of Hg in the presence of $HgS_{(s)}$. While the complexation of Hg with DOM has been shown to occur through an interaction of the Hg with reduced sulfur functional groups on the DOM, the influence of DOM on $HgS_{(s)}$ dissolution seems to be better correlated with the aromaticity of the DOM. This suggests that the interaction of Hg with DOM in the presence of sulfide is different than the interaction of Hg when sulfide is not present.

The interaction of Hg, sulfide and DOM could possibly be explained by a interaction of DOM with Hg-sulfide complexes or as a direct binding of Hg-sulfide to DOM. As a result of the hydrophobicity of neutral Hg-sulfide complexes, these complexes might be associating with nonpolar portions of DOM molecules. The correlation of $HgS_{(s)}$ dissolution with DOM aromaticity supports this type of interaction. Another possible interaction between Hg-sulfide complexes and DOM involves a direct binding of DOM to a Hg-sulfide complex. As mentioned earlier, HgS^0 likely exists as a hydrated complex, $HOHgSH^0$. While it is unlikely that DOM can displace the Hg-sulfide bond, the DOM could be interacting with the hydroxyl group on the $HOHgSH^0$ complex, resulting in a DOM-Hg-sulfide complex described by Equation 1.

$$HS\text{-}Hg\text{-}OH + HR = HS\text{-}Hg\text{-}R + H_2O \qquad \text{Equation 1}$$

In this equation, HR represents an organic compound with H being the proton on some functional group such as a thiol or carboxylic acid.

Our findings suggest that thermodynamic models based on complexation constants know to date overestimate the abundance of inorganic Hg-sulfide, because they do not take into consideration the interaction of Hg, sulfide and DOM. As a result of the correlation of Hg methylation with the abundance of neutral Hg-sulfide (Benoit et al. 2003), the influence of DOM on Hg complexation in the presence of sulfide could have significant implications on the formation of MeHg in aquatic systems. The uptake of Hg by sulfate reducing bacteria, the first step in the methylation of Hg, has been shown to be diffusively controlled, with only neutral Hg-sulfide complexes having the ability to enter the cell. Since diffusion rates are a function of neutral Hg-sulfide concentration, the decreased abundance of these complexes, as a result of the influence of DOM on the Hg speciation, could impact the uptake rate of Hg by bacteria. While small organic Hg complexes have be shown to be taken up by bacterial cells (Golding et al. 2002), the complexation of Hg with larger organic complexes inhibit cellular uptake (Barkay et al. 1997). It should be noted that rates of uptake of Hg, based on diffusion calculations, indicate that Hg enters bacterial cells at a rate several orders of magnitude faster than observed Hg methylation rates. Therefore, a reduction in the rate of Hg entering cells, as a result of the interaction of Hg with DOM under sulfidic conditions, might not significantly impact the methylation of Hg. The data presented here show that DOM needs to be

considered in Hg complexation in sulfidic environments, and that more work needs to be done to investigate how DOM may influence Hg methylation rates.

Chapter 5: Mechanisms controlling the interaction of Hg-sulfide complexes with dissolved organic matter

5.1. Introduction

The reactivity, mobility and bioaccumulation of mercury (Hg) in aquatic systems is controlled by its speciation, which varies with different environmental conditions. In most oxic environments, Hg speciation is driven by its complexation with organic matter (Benoit et al. 2001a; Hsu and Sedlak 2003; Lamborg et al. 2003; Ravichandran 2004). In oxygenated environments, complexation has been observed to influence the mobility of Hg through aquatic systems (Mierle and Ingram 1991; Driscoll et al. 1995), the distribution of Hg between particles and the dissolved phase (Gagnon et al. 1997; Regnell et al. 1997; Wallschlager et al. 1998b; Mason and Lawrence 1999b; Bloom et al. 2003), and also affects the uptake (Mason et al. 1996a; Barkay et al. 1997) and accumulation of Hg by aquatic organisms (Driscoll et al. 1995; Watras et al. 1995; Watras et al. 1998). Because Hg methylation occurs predominantly under anaerobic conditions (Benoit et al. 2003), the complexation of Hg in these environments, and the bioavailability of these complexes to Hg-methylation microorganism are of special interest. Under sulfidic conditions, the concentration of small, neutrally-charged dissolved phase Hg-sulfide complexes appear to control the rate of Hg uptake and methylation, by sulfate reducing bacteria (Benoit et al. 1999b; Benoit et al. 2001b; Benoit et al. 2001c). Until recently, it was thought that as a result of the strong association of Hg with sulfide, DOM did not

influence the Hg speciation under sulfidic conditions (Dyrssen and Wedborg 1991; Benoit et al. 2001a; Ravichandran 2004). However, an interaction between DOM and Hg under sulfidic conditions has been suggested from field studies (Kim and Sedlak 2005). Direct demonstrations of that interaction in the lab and field are described in detail in Chapters 3 and 4. Thermodynamic models that do not include a DOM-HgS (Benoit et al. 1999b) interaction will not provide accurate Hg speciation or neutral Hg-S complex concentrations under sulfidic conditions. However, the DOM-HgS interaction will remain difficult to model without understanding the mechanism of the interaction. In this chapter, that mechanism is investigated.

Mercury is classified as a B-type or soft sphere ligand indicating that its outer shell d-orbital electrons are highly polarizable and preferentially binds to ligands containing sulfur or nitrogen over oxygen containing ligands (Stumm and Morgan 1996). As a result, Hg forms strong complexes with inorganic and organic sulfur containing compounds with the inorganic complexes being thermodynamically favored. Several Hg-sulfide complexes can be present (HgS^0, $Hg(SH)_2^0$, $HgHS_2^-$, HgS_2^-) in the environment depending on the pH and sulfide concentration (Benoit et al. 2003). It should be noted, that while commonly written as HgS^0, quantum mechanical calculations indicate that this complex most likely is a hydrated complex ($HOHgSH^0$) (Tossell 2001). Thus, we will adopt this terminology in this chapter to reflect Tossell's results and in accordance with the notion that Hg(II) prefers to form linear complexes with two ligands.

Under oxic conditions, the complexation of Hg with DOM dominates the speciation in most environments. It is currently believed that the binding of Hg with

DOM occurs mainly through reduced sulfur functional groups on the DOM (Hesterberg et al. 2001; Haitzer et al. 2002; Ravichandran 2004). Mercury will also form complexes with other functional groups such as carboxylic acid groups (Xia et al. 1999), but these interactions are not strong enough to out compete the reduced sulfur groups on the DOM for Hg complexation at environmentally relevant Hg concentrations (Drexel et al. 2002; Haitzer et al. 2002). While the interaction of Hg with reduced sulfide groups on DOM has never been directly observed, as it has with solid organic matter (Xia et al. 1999), it has been suggested in several studies (Ravichandran et al. 1998; Benoit et al. 2001a; Drexel et al. 2002; Hsu and Sedlak 2003; Ravichandran 2004). Under natural conditions, the concentration of Hg is much less than the concentration of DOM reduced sulfur groups, the strong binding sites, therefore it is not likely that saturation of the strong sites occurs in nature (Haitzer et al. 2002). Stability constants for Hg-DOM complexes reported in the literature range from $10^{4.7}$ to greater than 10^{30}. However, the lowest values are probably underestimates resulting from studies done at high Hg concentrations. It is, therefore, thought that the stability constants that are the most accurate are between 10^{22}-10^{28} (Ravichandran 2004). These constants still span several orders of magnitude, most likely as a result of the heterogeneous nature of the DOM used in the different studies, and the different methods used to determine these constants (Ravichandran 2004).

Given the stability constants of Hg-DOM complexes and the complexation constants of Hg-sulfide complexes, thermodynamic models indicate that in the presence of measurable sulfide, Hg-sulfide complexes will dominate over Hg-DOM

complexes. Therefore, it is not expect that an interaction of Hg with DOM to occur in the presence of sulfide (Benoit et al. 2001a; Ravichandran 2004). Surprisingly, however, this type of interaction has been observed in waste water samples, porewater samples and laboratory prepared solutions using two different methods. Using C-18 column extractions with a competitive ligand, DOM was shown to reduce the concentration of inorganic Hg-sulfide complexes. In that study, it could not be determined whether DOM was interacting directly with Hg-sulfide complexes or if the observation was a methodological artifact (Hsu-Kim and Sedlak 2005). However, direct interaction was confirmed using octanol-water partitioning and ultrafiltration in which DOM was shown to reduce the formation of Hg-sulfide complexes in various natural waters and laboratory solutions (Chapter 4). While it is clear that DOM influences the formation of Hg-sulfide complexes, the mechanism driving this reaction is not understood.

A similar interaction in which DOM increases the dissolution and inhibits the formation of solid $HgS_{(s)}$ has been observed (Ravichandran et al. 1998; Ravichandran et al. 1999; Waples et al. 2005). In these studies, several DOM isolates and small model organic ligands were used to examine the interaction of DOM with solid $HgS_{(s)}$. The information gained from these studies provides a starting point for investigating the interaction of DOM with dissolved phase Hg-sulfide complexes. The aromaticity, molecular weight and ultraviolet absorption of the DOM seemed to be the most important characteristics driving the interaction between DOM and $HgS_{(s)}$ (Ravichandran et al. 1998; Waples et al. 2005). The reduced sulfur content of the DOM appeared to be important in reducing the precipitation of $HgS_{(s)}$ in one study

(Ravichandran et al. 1999), but this was not observed in the other studies (Ravichandran et al. 1998; Waples et al. 2005). Model ligands were also used in these studies, and it was shown that only mercaptoacetic acid, also known as thioglycolic acid, was able to elicit the same response as the DOM (Ravichandran et al. 1998; Ravichandran et al. 1999). EDTA, acetic acid and salicylic acid did not influence these processes significantly, while calcium was shown to suppress the influence of DOM on these processes (Ravichandran et al. 1998; Ravichandran et al. 1999). One potential mechanism, proposed by these authors, for the interaction of DOM with solid $HgS_{(s)}$ is that DOM binds to the solid surface, causing dissolution and subsequent release of a Hg-DOM complex. This interaction seems unlikely, however, because DOM is a weaker ligand for Hg relative to sulfide. DOM should not have the ability to out-compete sulfide for Hg, either in solution or on the surface of the $HgS_{(s)}$ (Ravichandran et al. 1998). Another proposed mechanism is DOM-driven redox reactions are occurring at the solid surface, resulting in the release of Hg (Ravichandran et al. 1998; Waples et al. 2005). The ability of calcium to negate the affects of DOM was attributed to either a change in the electrostatic interaction of the DOM with the surface of the $HgS_{(s)}$ or a complexation of the calcium with carboxylic acid functional groups on the DOM and in turn reducing the interaction of the DOM with the $HgS_{(s)}$.

In Chapter 4 it was shown that DOM influences the speciation of Hg under sulfidic conditions. In order to investigate the mechanism controlling this interaction, several experiments were conducted to examine the interaction of Hg with DOM under sulfidic conditions. Using octanol-water partitioning (D_{ow}) and centrifugal

ultrafiltration, the interaction of Hg with model organic ligand and DOM isolates was investigated to elucidate the interaction of Hg-sulfide complexes with DOM. From these studies, it was determined that only neutral Hg-sulfides interact with DOM. Conditional stability constants were determined for this interaction and these constants were applied to thermodynamic models to calculate the speciation of Hg under sulfidic conditions.

5.2. Methods

5.2.1. Octanol-water partitioning overview

D_{ow} extractions take advantage of the differences in the partitioning of Hg complexes into octanol. The partitioning coefficient, K_{ow}, (Equation 1) is a measure of the tendency of a Hg complex to partition into octanol and is a function of the hydrophobicity of the complex. In Equation 1, HgL_{aq} and HgL_{oct} represent the concentration of a specific Hg complex (HgL) in the aqueous and octanol phase, respectively. In sample solutions, several Hg complexes are likely present and, therefore, the measured distribution of Hg between the aqueous and octanol phases is referred to as the overall partitioning coefficient (D_{ow}), which is defined by Equation 2. Hg_{aq} and Hg_{oct} are the total concentrations of Hg in the two phases and in most solutions represent several different Hg complexes. Equation 3 can be used to relate measured D_{ow} values and known K_{ow} values with the fractional abundance (α_i) of the different complexes in the solution. Table 3.1 list known K_{ow} values for several Hg complexes. For this study, it is important to note the difference in the K_{ow} for neutral Hg-sulfide complexes (K_{ow}= 72) and Hg-DOM complexes (K_{ow} = 1.7-3.3). Several

studies have used octanol-water partitioning methods to examine Hg complexation and bioavailability in artificial solutions (Mason et al. 1996a; Benoit et al. 1999a; Jay et al. 2000; Benoit et al. 2001a). An extension of the method for use in low-Hg natural waters is given in Chapter 3.

$$K_{ow} = HgL_{oct}/HgL_{aq} \quad (1)$$

$$D_{ow} = Hg_{oct}/Hg_{aq} \quad (2)$$

$$D_{ow} = \Sigma\alpha_i(K_{ow})_i \quad (3)$$

5.2.2. Model ligand interactions

In order to understand the interaction that occurs between Hg-sulfide complexes and DOM, model ligands were used to examine if specific functional groups on the DOM might be responsible for suppressing the concentration of free Hg-sulfide complexes. Specifically, the D_{ow} of Hg in solution with EDTA or thioglycolate and sulfide was examined. To examine the concentration dependence of the interactions, thioglycolate and EDTA concentrations were varied, while sulfide was held constant at approximately 20 μM. The D_{ow}'s of Hg thioglycolate or EDTA complexes, without sulfide present, were also measured. All solutions were prepared in an anaerobic chamber using deoxygenated, pH 6 phosphate buffer (0.04 M) in order to maintain an environmentally relevant pH. The experiments were performed using an enriched Hg stable isotope, Hg-199, added to obtain approximately 1.2 ng/L Hg-199 and all solutions were equilibrated overnight before D_{ow} extractions were

preformed. Extractions were conducted for 2 hours using 80 mL of solution and 20 mL of octanol. Wall losses were assessed by carrying out separate extractions without octanol. As mentioned in Chapter 3, the interface between the aqueous and octanol layer was not always clear as a result of the formation of an emulsion between the layers, which was hard to separate. The emulsion was included in the aqueous layer whenever possible but in some experiments it was not possible to separate the emulsion from the octanol layer. Details of the D_{ow} extraction can be found in Chapter 3.

5.2.3. DOM concentration gradient study

To examine if there is a DOM concentration dependence on the interaction of Hg, sulfide and DOM, D_{ow} extractions were conducted on solutions containing different concentrations of Suwannee River natural organic matter, purchased from the International Society of Humic Substances, in the presence and absence of sulfide. Some characteristics of this DOM isolate are shown in Table 4.2. Three solutions were prepared at DOM concentrations of 0.48, 4.8, and 14.6 mg C/L using degassed pH 6 phosphate buffer (0.04 M). Each solution was split and sulfide was added to half the sample to reach an approximate sulfide concentration of 20 μM. Enriched Hg-199 was added to the solutions at approximately1.2 ng/L Hg-199.

5.2.4 DOM/calcium interaction study

Calcium has been shown to inhibit the influence of DOM on the precipitation and dissolution of solid $HgS_{(s)}$ (Ravichandran et al. 1998; Ravichandran et al. 1999; Waples et al. 2005), therefore, the influence of calcium on the dissolved phase interaction of Hg, sulfide and DOM was examined. Calcium is known to interact with carboxylic acid functional groups of DOM (Benedetti et al. 1995), consequently, adding calcium to solutions containing Hg, sulfide and DOM could provide insight into the mechanism controlling the interaction of DOM on the complexation of Hg under sulfidic conditions. A series of solutions were also prepared in degassed, pH 6 phosphate buffer using Suwannee River DOM (14.6 mg C/L), approximately 20 μM sulfide and several calcium concentrations (10^{-3}, 10^{-4}, 10^{-5} M $CaCl_2$). All solutions were equilibrated overnight and D_{ow} extractions were prepared as described above.

5.2.5. Ultrafiltration overview

Octanol-water extractions only have the ability to detect the presence of neutral Hg-sulfide complexes, therefore, ultrafiltration experiments were used to determine if DOM influences the concentration of both charged and uncharged Hg-sulfide complexes. For the ultrafiltration experiments, Millipore Amicon Ultra-15 centrifugal filters were used with a nominal molecular weight limit of 5000 Da. The filtration was completed by centrifuging the filters containing the sample solution at 4000 rpm on a Sorvall RT 6000 swinging bucket centrifuge. To clean new filters, Milli-Q water was passed through each filter five times to ensure the removal of any residual organic complexes, such as glycerol, which is used in the production of the

filters. For all experiments, 15 mL of solution was filtered and the Hg in the solutions passing through the filter (filtrate) and the solution remaining above the filter was analyzed. The Hg complexes in the filtrate were less than 5000 Da while the Hg complexes remaining above the filter were greater than 5000 Da. For all experiments using the centrifugal ultrafilters, Hg enriched in a single stable isotope (Hg-199) was used, and as a result, Hg contamination associated with the filters was not a significant concern. In order a examine Hg sorption to the filters, Hg remaining on the filters was examined. After the filtrate and the solution above the filter was removed, 15 mL of a bromine monochloride solution (0.13M KBr; 0.06M $KBrO_3$) was added to the filters and held for a minimum of 24 hours. The amount of Hg extracted in this solution was determined. A detail description of the applicability of this method is presented elsewhere (Chapter 4).

5.2.6. DOC and sulfide concentration gradient

Two experiments were conducted to examine the interaction of Hg with Suwannee River DOM using ultrafiltration. In the first experiment, the sulfide concentration was held constant (7.8 ± 0.6 μM) and three DOM concentrations were used (5.9, 12.1, and 19.2 mg C/L). In the second experiment, the DOM concentration was held constant (11.7 mg C/L) and the sulfide concentration was varied (2.9, 23.21 and 269 uM). In both experiments, the solutions were prepared in deoxygenated, pH 6 phosphate buffer (0.04 M) and equilibrated overnight before separation. Enriched Hg-199 was added to all solutions to obtain a Hg-199 concentration of approximately 24 ng/L.

In all ultrafiltration experiments, the DOM was prepared so that only the greater than 5000 Da fraction was used in the experiments to ensure that all the Hg associated with the DOM would remain above the filter after the filtration. An initial solution containing 17 mg C DOM was dissolved in 150 mL phosphate buffer. This solution was ultrafiltered and the DOM greater than 5000 Da was diluted in phosphate buffer and filtered through a glass fiber filter. This solution was than diluted to reach the desired DOM concentrations. The validity of this method to separate organically associated Hg greater than 5000 Da and inorganic Hg complexes were presented in Chapter 4. As mentioned in that Chapter, the Hg remaining above the filter after centrifugation is assumed to be DOM associated Hg.

5.2.7. Analytical methods

Total Hg was analyzed by stannous chloride reduction followed by purging and trapping on gold beads (Gill and Fitzgerald 1987; Bloom and Fitzgerald 1988). Inductively-coupled plasma mass spectrometer (ICP-MS) was used in place of cold vapor atomic fluorescence as a detector, thus allowing analysis of the enriched stable Hg isotope used in the experiments to be separated from background Hg (Hintelmann and Evans 1997b; Hintelmann and Ogrinc 2003). A Hewlett Packard 4500 ICP-MS was used for all analyses. To oxidize any organic matter in aqueous samples, bromine monochloride was added for a minimum of twenty-four hours prior to analysis. Bromine monochloride was added at a level to ensure that there was an excess of this reagent in the samples, as determined by the presence of orange coloration. Just prior to analysis hydroxylamine hydrochloride was added to the

samples to reduce any excess bromine monochloride. Background Hg concentrations were assessed by analysis of Hg-202. Experiments were conducted using enriched Hg-199 (91.95% pure) (Oak Ridge National Laboratories) which upon addition to the experimental solution was in the form of a Hg-chloride complex. Stable isotopes of Hg and ICP-MS detection have been used in several studies in our lab (Heyes et al. 2004; Kim et al. 2004; Heyes et al. in press; Kim et al. in press; Sunderland et al. in press; Whalin and Mason in press). A detailed description of both the analysis and calculations associated with using an enriched isotope are shown elsewhere (Hintelmann and Ogrinc 2003).

A solid state ion selective electrode and a reference electrode (Thermo Electron Corporation) was used to analyze for sulfide (Eaton 1998). Sample and standards were preserved in freshly prepared sulfide antioxidant buffer (SAOB) and analyzed within 4 hours. DOC samples were analyzed, using a Shimadzu TOC-5000 analyzer, by the Nutrient Analytical Services Laboratory at the Chesapeake Biological Laboratory.

5.2.8. Thermodynamic modeling

Speciation calculations were performed using the program MINEQL+ (Environmental Research Software, Hallowell, ME, USA). The formation constants for Hg-sulfide complexes (Table 1.1) (Benoit et al. 1999a) and a complexation constant for Hg to DOM based on the molar concentration of DOM, as presented in Benoit et al. 2001 (Benoit et al. 2001a), were added into the MINEQL+ database. All other complexation constants were used directly from the MINEQL+ database.

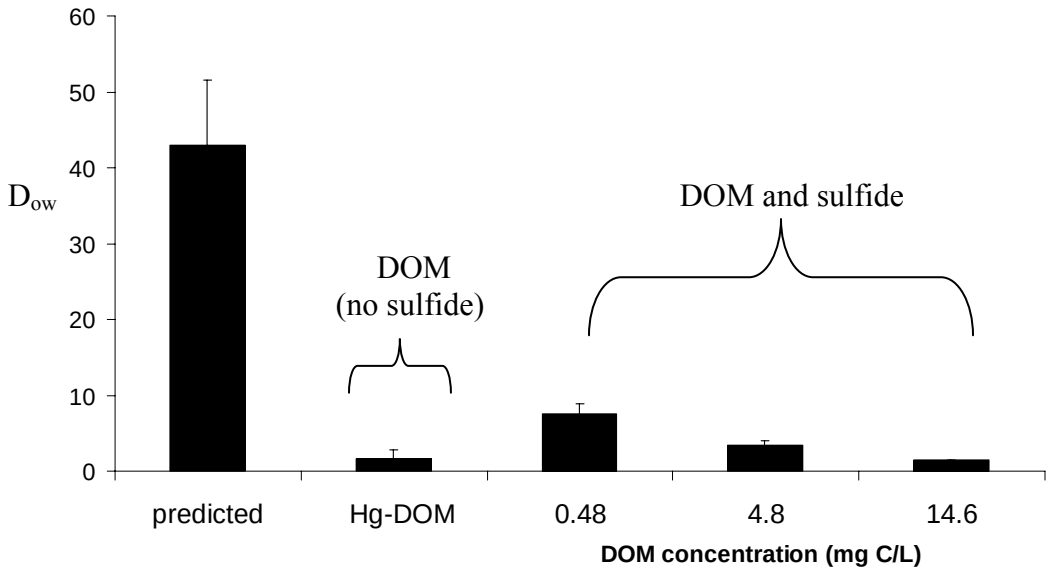
Thermodynamic modeling was used to predict the speciation of Hg in the different experimental solutions and subsequently the speciation was used to calculate the predicted D_{ow}'s. These values are predictions based on current thermodynamic information, which does not include the interaction of Hg with DOM in the presence of sulfide.

5.3. Results/Discussion

5.3.1. Dow DOM concentration gradient

An interaction between Hg, sulfide and DOM was observed using D_{ow} extractions at three different concentrations of Suwannee River DOM in the presence of sulfide (Figure 5.1). The dominant Hg species, calculated using thermodynamic modeling without the inclusion of the interaction of Hg-sulfide with DOM, and determined from the average pH (6.10 ± 0.01) and the average sulfide concentration (22 ± 2 μM) of the three solutions, would be $HOHgSH^0$ (49.6 %), $Hg(SH)_2^0$ (10.0 %) and $HgHS_2^-$ (40.0 %). If DOM did not influence the formation of Hg-sulfide complexes, the predicted D_{ow} for all three solutions would be 43 ± 9. This value, determined using Equation 3, the thermodynamically predicted speciation of Hg and the known K_{ow} for the different Hg-sulfide complexes, is much higher than the values measured in all three solutions containing DOM (Figure 5.1). The influence of DOM on the formation of neutral Hg-sulfide complexes is dependent on the concentration of DOM, with a stronger interaction, as indicated by a lower measured D_{ow}, at higher DOM concentrations. The low D_{ow}'s measured in solutions containing Hg, DOM and

Figure 5.1: Affect of Suwannee River DOM concentration on the partitioning of Hg into octanol in the presence of 20 μM sulfide. The predicted Dow assumed that DOM did not affect the speciation of Hg in the presence of sulfide. In the DOM (no sulfide) treatment, Hg-DOM was the dominant complex. The error bars represent the standard deviation of three replicate samples.



sulfide indicate that the concentration of neutral Hg-sulfide complexes, which have K_{ow}'s of 72 ± 15 (Chapter 3), are much less than predicted by thermodynamic models, supporting an interaction between Hg, sulfide and DOM. It is reasonable to assume that a complex containing Hg, DOM and sulfide will partition poorly into octanol, and to a similar extent as a Hg-DOM complex, since the partitioning is likely driven by the size and charge of the DOM portion of the complex. The D_{ow} for Hg complexed to Suwannee River DOM, the DOM used in these experiments, is 1.7 ± 1.1 (Chapter 3) and the measured D_{ow} for Hg in solutions containing Hg, sulfide and

DOM are similar to this D_{ow} (Figure 5.1). This supports the formation of a complex between Hg, sulfide and DOM. Since charged Hg-sulfide complexes do not partition into octanol ($K_{ow} < 0.01$) (Benoit et al. 1999a), an interaction of these complexes with DOM would not be detected using D_{ow} extractions. This method only has the ability to determine the abundance of neutral Hg-sulfide complexes, so from this data, it can only be determined that the concentration of neutral Hg-sulfide complexes are lower than predicted by thermodynamic models as a result of an interaction with DOM.

5.3.2. Dow model ligands and calcium interaction experiments

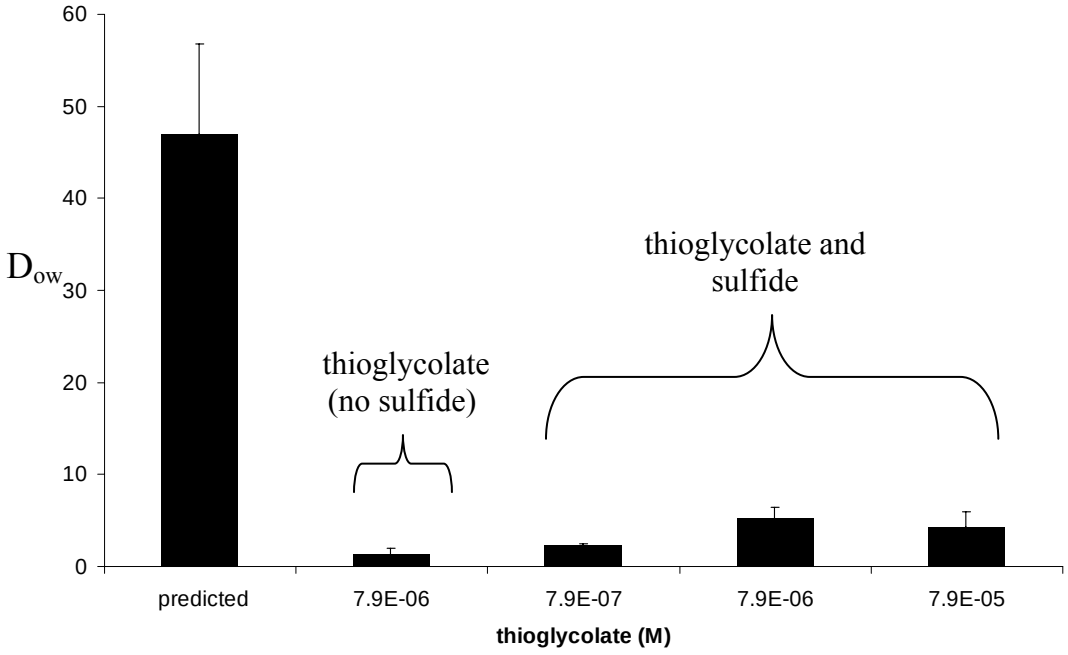
D_{ow} extractions were also used to examine the interaction of small organic ligands with Hg-sulfide complexes as a means to determine if the interaction between dissolved phase Hg, DOM and sulfide is similar to the interactions observed between solid phase $HgS_{(s)}$ and DOM. In experiments with $HgS_{(s)}$, it was shown that thioglycolate elicited the same response as DOM in that it reduced the precipitation and enhanced the dissolution of $HgS_{(s)}$ (Ravichandran et al. 1998; Ravichandran et al. 1999). Other small model organic ligands, such as EDTA, did not influence the precipitation and dissolution of $HgS_{(s)}$. These studies also found that when Ca^{2+} was included with DOM in the experimental solutions, the influence of DOM on dissolution of $HgS_{(s)}$ was negated. It was suggested that Ca^{2+} either complexed with the negative surface sites of the solid $HgS_{(s)}$ or interacted with the carboxylic acid functional groups on the DOM causing a change in the interaction of the DOM with

the $HgS_{(s)}$. Given the results presented later in the chapter, we suspect that the interaction of calcium was with the solid phase and not the DOM.

D_{ow} extractions on solutions containing thioglycolate ($HSCH_2COO^-$) as a model ligand were used to determine if the interaction between Hg, sulfide and DOM can be attributed to an interaction with carboxylic acid or thiol containing functional groups on organic matter. The average pH (6.12 ± 0.7) and sulfide concentration (17.6 ± 2.0 μM) were used to predict the speciation of Hg in the experimental solution, which was dominated by neutral species (~60% HOHgSH and ~10% $Hg(SH)_2$). Based on the complexation constants of Hg to thioglycolate, at the thioglycolate and sulfide concentrations used in the experiment, Hg should not form Hg-thioglycolate complexes. The D_{ow} for Hg in a solution containing only thioglycolate was 1.4 ± 0.6. In the three solutions containing thioglycolate and sulfide, the predicted D_{ow} was 47 ± 10 if there was no interaction of the thioglycolate with the Hg-sulfide complexation. The measured D_{ow} in the three solutions ranged from 2.4 to 5.3 (Figure 5.2) indicating that the presence of thioglycolate is reducing the concentration of free neutral Hg-sulfide complexes in solution. Since thioglycolate contains both thiol and carboxylic acid functional groups, it can not be determined from these results if a specific functional group is driving this interaction.

In order to determine if an interaction between Hg-sulfide complexes and carboxylic acid functional groups could be important in the interaction between Hg, sulfide and DOM, solutions were prepared at three concentration of EDTA (2×10^{-7}, 2×10^{-6}, and 2×10^{-5} M). The average sulfide concentration in the three solutions, 20 ± 2 μM, and the average pH, 6.06 ± 0.01, was used to predict the speciation. The

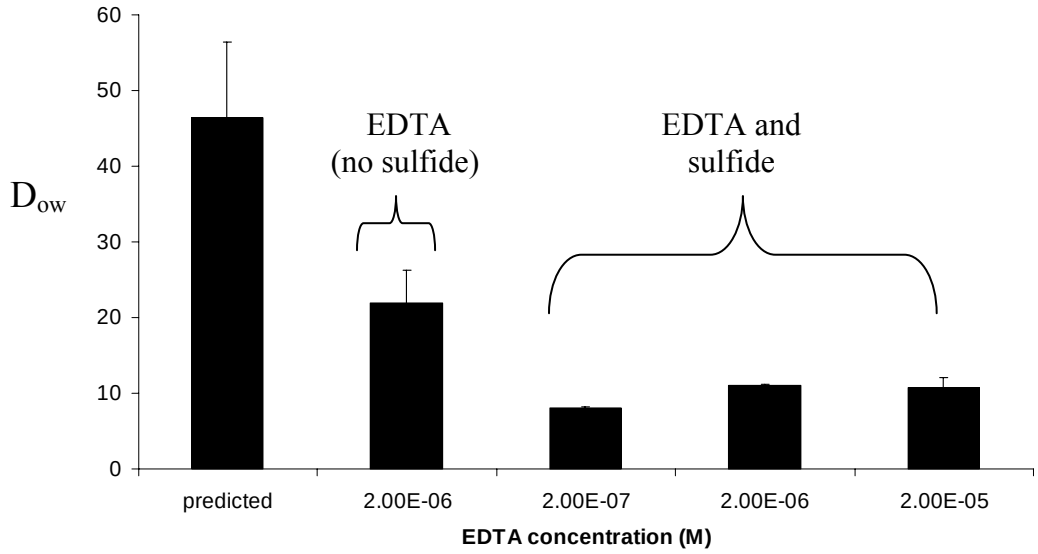
Figure 5.2: Effect of thioglycolate on Hg partitioning into octanol in the presence of 20 μM sulfide. The predicted D_{ow} assumed that thioglycolate did not affect the speciation of Hg in the presence of sulfide. The thioglycolate treatment contained no sulfide resulting in the Hg-thioglycolate complex dominating the Hg speciation. The error bars represent the standard deviation of three replicate samples.



measured D_{ow}'s of the three solutions are shown in Figure 5.3, along with the D_{ow} for a solution containing EDTA (2×10^{-6} M) without sulfide.

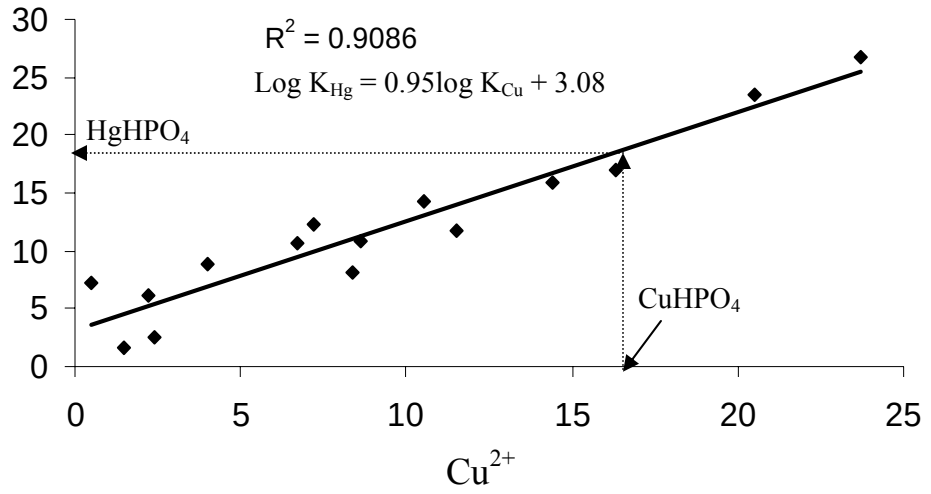
The measured D_{ow} for Hg in the solution containing no sulfide ($D_{ow} = 22 \pm 4$) is higher than the previously reported values for Hg-EDTA solutions ($D_{ow} = 0.1$) (Benoit et al. 2001a). Using the reported complexation constants of Hg to EDTA, the calculated speciation of Hg in this solution indicates that $Hg\text{-}EDTA^{2-}$ should comprise 100% of the Hg speciation and this complex would not be expected to

Figure 5.3: Affect of EDTA on Hg partitioning into octanol in the presence of 20 μM sulfide. The predicted D_{ow} assumed that EDTA did not affect the speciation of Hg in the presence of sulfide. In the EDTA (no sulfide) treatment the dominant complex was Hg-EDTA. The error bars represent the standard deviation of three replicate samples.



partition into octanol. One potential explanation for the results obtained, is the possibility that phosphate, added to the solution as a buffer, might be forming a neutral complex with the Hg and, therefore, eliminating Hg-$EDTA^{2-}$ as the dominate complex. While complexation constants of Hg to phosphate are not known, they can be estimated from the relationship of the complexation constants of Hg and Cu^{2+} with different ligands (Figure 5.4). From this relationship, the log of the complexation constant for the formation of $HgHPO_3^0$ was estimated as 18.8. The speciation of Hg, when the potential interaction of Hg with phosphate is include, still suggests that Hg-$EDTA^{2-}$ is the dominate complex. While there is uncertainty in the complexation

Figure 5.4: Relationship between the log of the complexation constants of Cu^{2+} and Hg^{2+} for several inorganic and small organic ligands (complexation constants taken from Stumm and Morgan, 1996). Using this relationship the formation constant for $HgHPO_4$ can be predicted from the formation constant of $CuHPO_4$ (log K = 16.5).



constant of $HgHPO_3^0$, this constant would have to be 5 orders of magnitude higher, which in unlikely, for the formation of $HgHPO_3^0$ to be important.

Another potential complex that might be forming is HgH_2EDTA, which would significantly partition into octanol since it is a neutral complex. Based on thermodynamic calculations, at pH 6, the pH the experiments were conducted, approximately 50% of the EDTA in solution would exist as H_2EDTA^{2-}. While a thermodynamic constant is not known for the formation of HgH_2EDTA, it is not unreasonable to believe that this complex might be forming, especially give the abundance of H_2EDTA^{2-} at the pH of the experiments. While there is no way of determining if HgH_2EDTA is present in the experimental solutions, the formation of this complex would explain the higher than expected D_{ow} measured for the solution containing Hg and EDTA.

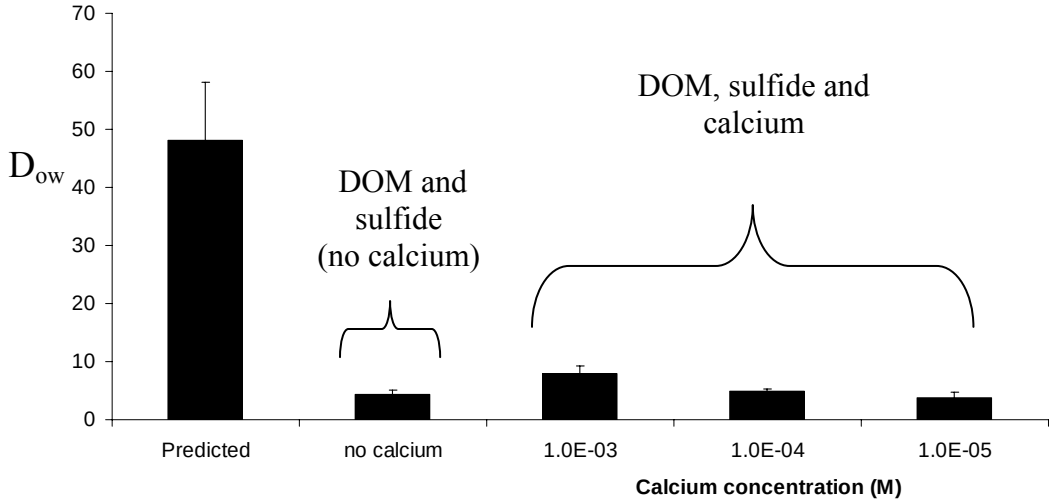
Another possible explanation for the larger measured D_{ow} in this study relative to the previously measured D_{ow} for similar solutions (Benoit et al. 2001a) is a difference in the D_{ow} extraction used in the two studies. As mentioned earlier, the separation between the octanol and aqueous layer was not always clear as a result of an emulsion forming between the layers. While in this study, it was attempted to include this emulsion layer in the aqueous phase, it was not always possible to separate the emulsion from the octanol. Since the amount of Hg in the octanol was determined by difference between the amount of Hg measured in the whole sample and the Hg in the aqueous fraction, any Hg in the emulsion layer that was not included in the aqueous fraction would have been assumed to be in the octanol fraction. This resulted in a measured D_{ow} higher than if the Hg in the emulsion was not considered or included in the aqueous layer. Since a radioactive Hg isotope was used in Benoit et al. 2001a, a study using Hg concentrations three orders of magnitude higher than used in our study, small subsamples were collected from both the aqueous and octanol phase for analysis. Any Hg in the emulsion layer would not have been considered in these experiments. The recovery of the added Hg was not reported in that study, therefore the loss of Hg as a result of inclusion in the emulsion layer can not be accessed. It should be noted, that in another study that collected only a small subsample of the aqueous and octanol layer (Benoit et al. 1999a), 96% of the added Hg was not recovered. This was attributed to the precipitation of Hg and the sorption of Hg to the walls of the extraction containers, but another possible loss term could be the incorporation of Hg in the emulsion layer. Since Hg-$EDTA^{2-}$ is a charged complex it would not be expected to partition into octanol, but it is possible

that this complex is included in the emulsion layer. While measured D_{ow}'s for Hg-EDTA solutions differ between studies, this difference does not affect the overall interpretation of the data in this study.

Since the complexation of Hg is stronger with sulfide than with EDTA, in the presence of sulfide and EDTA, all of the Hg should be present as Hg-sulfide complexes and the predicted D_{ow} would be 46 ± 10. The measured D_{ow} for Hg in the three solutions were all less than predicted if no interaction occurred between the EDTA and the Hg-sulfide complex and less than that of Hg-EDTA alone (Graph 5.3). This suggests that EDTA is suppressing the formation of neutral Hg-sulfide complexes. Since the measured D_{ow} of the solution containing Hg, EDTA and sulfide is less than both the K_{ow}'s for Hg-$EDTA^{2-}$ and neutral Hg-sulfide complexes, these results support an interaction between Hg, sulfide and EDTA. Both thioglycolate and EDTA were able to mimic the interaction of DOM with Hg-sulfide, suggesting that if the interaction is driven by functional groups on the DOM, carboxylic acid functional groups and potentially thiol functional groups could be responsible for this interaction.

Calcium, as $CaCl_2$, was added to solutions of Hg, sulfide and DOM to investigate whether Ca^{2+} increased the D_{ow} for Hg, which would suggest an inhibition of HgS-DOM complex formation. As in other experiments, the measured D_{ow} for Hg in the control sulfide and DOM mixture was roughly an order of magnitude below that predicted based on models without a HgS-DOM interaction (Figure 5.5). The measured D_{ow} in a solution without Ca^{2+} was 4.3 ± 0.7 and there was no statistical significant increase ($p = 0.01$) in the D_{ow} with the addition of 10^{-4} M and 10^{-5} M Ca^{2+}.

Figure 5.5: The effect of calcium on the interaction of Suwannee River DOM with Hg in the presence of sulfide, as measured by the D_{ow} of Hg. The predicted D_{ow} value assumes that DOM and calcium do not affect the Hg-sulfide speciation. The no calcium treatment contained only DOM and sulfide while the other treatments contained DOM, sulfide and calcium. All solutions contained 14.6 mg C/L DOM. Error bars are the standard deviation of three replicates.



A slight increase in the D_{ow} was observed in the 10^{-3} M Ca^{2+} treatment ($p = 0.01$) but the measured D_{ow} of 8.0 ± 3 was still considerably below the predicted D_{ow} of 48.1 ± 10.0 if DOM was not influencing the formation of Hg-sulfide complexes. Thus, the effect of Ca^{2+} on the concentration of neutral HgS species was either nonexistent or minimal. Since Ca^{+} ions binds to carboxylic acid groups (Benedetti et al. 1995), an inhibition in the interaction of Hg-sulfide with DOM in the presence of Ca^{2+} would suggest that the carboxylic acid groups are responsible for the interaction. This was not observed in the experiments suggesting carboxylic acid functional groups on the DOM might not be driving the interaction of DOM with Hg-sulfide.

While information gleaned from studies examining the interaction of DOM with solid phase $HgS_{(s)}$ provided a starting point in the understanding of the interaction between dissolved phase Hg, DOM and sulfide, the model ligand experiment presented here suggest that the mechanism controlling these two interactions might be different. Thioglycolate appears to mimic DOM in its interaction with both solid phase $HgS_{(s)}$ and dissolved phase speciation suggesting that thiol ligands might be important in both of these processes. While EDTA behaved similarly to DOM, in that it reduced the formation of neutral Hg-sulfide complexes, in the dissolved phase speciation of Hg, it did not influence the $HgS_{(s)}$ solid phase dynamics. The addition of calcium into solution did not influence the interaction of Hg, DOM and sulfide in dissolved phase experiments while calcium did affect the interaction of DOM with $HgS_{(s)}$. Since similar results were not observed with the model organic ligand and calcium additions between the dissolved and solid phase interaction of Hg and sulfide, it appears that DOM might be influencing these processes differently. The ability of EDTA to mimic DOM in the decreased formation of Hg-sulfide complexes suggests that carboxylic acid functional groups might be important in controlling this interaction under the experimental conditions. However Ca^{2+} did not influence the interaction of DOM with Hg-sulfide complexation, which questions the importance of carboxylic acid functional groups. If the inhibition of the observed interaction of DOM with $HgS_{(s)}$ is a result of Ca^{2+} interacting with the DOM, similar interaction would be expected in the dissolved phase. However, if the Ca^{2+} interacted with the solid $HgS_{(s)}S$ surface rather than the DOM, the lack of effect of Ca^{2+} on the interactions being studied here would be

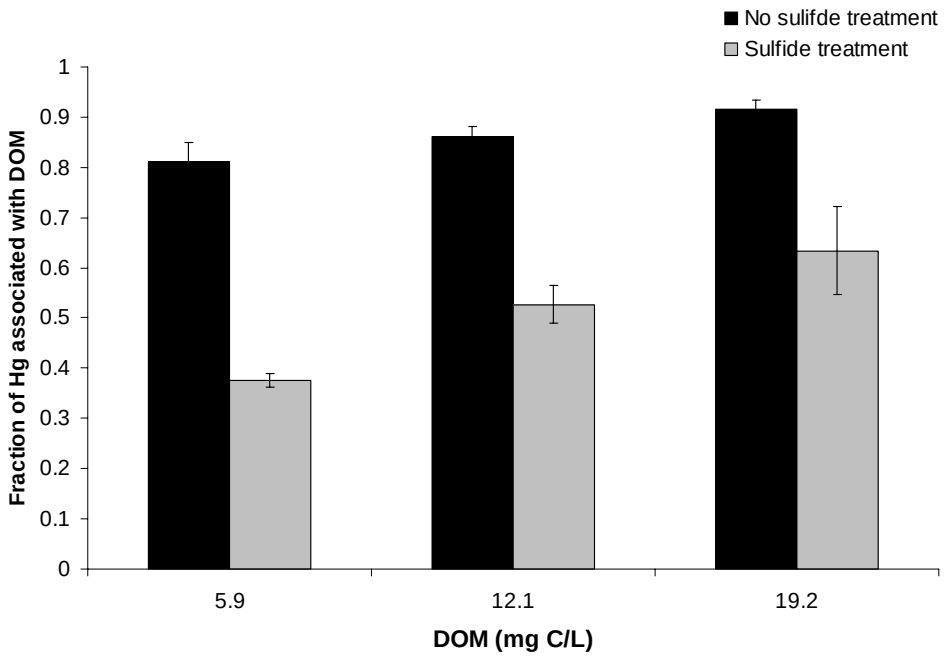
expected. It should be noted that in the studies examining the solid phase $HgS_{(s)}$, the molecular weight and aromaticity of the DOM was correlated with the dissolution of $HgS_{(s)}$ (Waples et al. 2005). If the aromaticity of the DOM is more important than the functional groups on the DOM, the observed interactions using model ligands might not be a result of the same interaction which is occurring with the DOM.

5.2.3. Ultrafiltration DOM and sulfide gradient

Several experiments were conducted using centrifugal ultrafiltration to understand the interaction of Hg-sulfide complexes with DOM. Using ultrafiltration, measurements can be made to determine if the Hg in a solution is contained in a complex greater or less than 5000 Da. For all experiments, the DOM used was greater than 5000 Da, and therefore, any Hg associated with DOM would remain above the filter after filtration. Small Hg complexes, such as inorganic Hg-sulfide complexes would either pass through or sorb to the filter. Validation of this method for the examination of Hg complexation has previously been shown (Chapter 4). The data from the D_{ow} experiments indicated that an interaction between DOM and neutral Hg-sulfide complexes occurs but a similar investigation of an interaction of DOM and negatively charged Hg-sulfides could not be done using D_{ow} extractions. Ultrafiltration provides a means to examine the interaction of DOM with both positively charged and uncharged Hg-sulfide complexes.

The increased interaction between Hg, sulfide and DOM with increasing DOM concentration, observed using D_{ow} extractions, was supported by ultrafiltration experiments. Ultrafiltration experiments were initially used to examine the

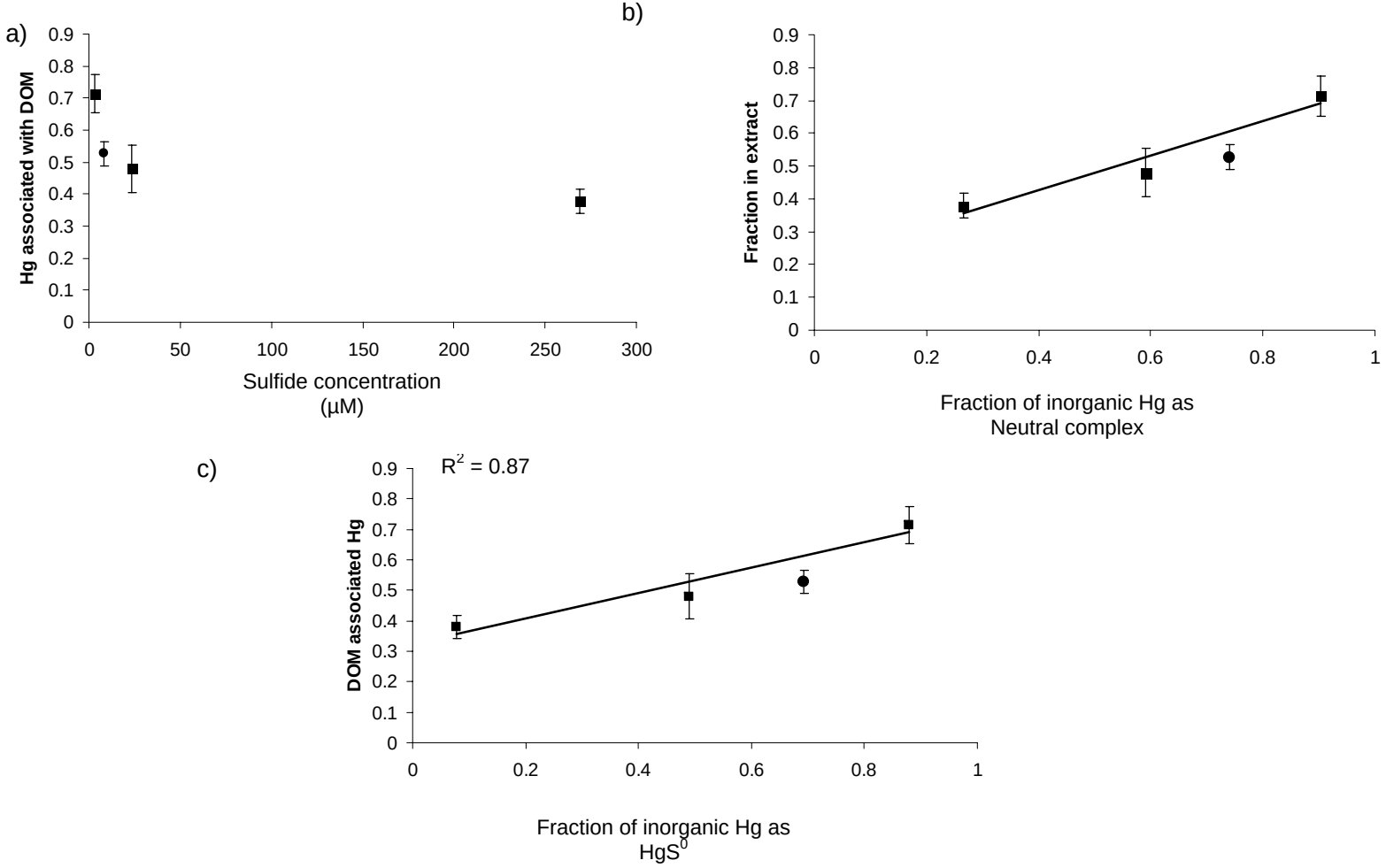
Figure 5.6:Ultrafiltration results for solutions containing DOM (black bars) and DOM and sulfide (gray) bars at three concentrations of Suwannee River DOM. For each treatment the fraction of the Hg associated with DOM is shown. The error bars represent the standard deviation of three replicates.



dependence of the interaction of Hg, sulfide and DOM on the DOM concentration using solutions containing Hg, sulfide and a gradient of DOM concentrations (Figure 5.6). As shown in Chapter 4, in the presence of DOM, greater than 80% of the Hg remained above the filter when sulfide was not present indicating that up to 20% of the Hg-DOM complex is adsorbing to the filter. In these solutions 100% of the mercury should be complexed to DOM. When sulfide is added to the solutions, the amount of Hg associated with DOM was reduced, but even at the lowest DOM concentrations (5.9 mg C/L) more than 30% of the Hg is associated with DOM.

Ultrafiltration was also conducted on a series of solutions containing a range of sulfide concentration (~3-270 μM sulfide) at a fixed DOM concentration (11.7 mg C/L). As the sulfide concentration increases, the abundance of charged inorganic Hg-sulfide complexes increases. By examining the effect of DOM on the Hg-sulfide abundance over a sulfide gradient, the relative importance of charged and uncharged species can be examined. As the sulfide concentration increases, the amount of Hg associated with DOM decreased (Figure 5.7a). Increasing sulfide concentrations corresponds with a decrease in the abundance of neutral Hg-sulfide complexes (Table 5.2). If DOM was interacting with both charged and neutral Hg sulfide complexes to the same extent, the amount of Hg associated with DOM would not change over a range of sulfide concentrations. A decrease in the Hg associated with DOM was observed with increasing sulfide concentration, but this relationship was not linear (Figure 5.7a). The decrease in the association of Hg with DOM as the sulfide increases could also indicate that only the neutral Hg-sulfide complexes are interacting with the DOM, since the abundance of theses species decrease with increasing sulfide. A better correlation is obtained by plotting the fraction of Hg associated with DOM versus the fraction of Hg present as neutral complexes (sum of $HOHgHS^0$ and $Hg(HS)_2^0$) (Figure 5.7b). A relationship is also obtained by plotting the fraction of Hg associated with DOM versus the fraction of Hg present as $HOHgSH^0$ (Figure 5.7c). These relationships are based on only four data points. Three of the data points are from the experiment in which the DOM concentration was held constant (11.7 mg C/L) and the sulfide concentration was varied. The other

Figure 5.7: Mercury associated with Suwannee River DOM after ultrafiltration The DOM concentration was 11.9 mg C/L (squares) or 12.1 mg C/L (circles). The Hg associated with DOM is plotted versus the sulfide concentration (a), the concentration of neutral Hg-sulfide (sum of $HOHgHS^0$ and $Hg(SH)_2{}^0$)(b) and the concentration of $HOHgSH^0$(c)



point is the middle DOM concentration (12.1 mg C/L) in the ultrafiltration experiments examining three DOM concentrations at a constant sulfide concentration. Since the concentration of DOM influences the fraction of Hg associated with DOM (Figure 5.6) only solutions containing similar DOM concentrations can be used to examine the relationship between the formation of the Hg-sulfide-DOM complex and sulfide concentration. While only four data points are available, the observed relationship suggests that the interaction of Hg-sulfide complexes with DOM is driven by an interaction of the neutral complexes. Although, from this data it is not clear if this interaction is driven by $HOHgSH^0$ or if $Hg(HS)_2^0$ and $HOHgSH^0$ are both involved.

The data from the D_{ow} extractions of laboratory solutions containing Hg, sulfide and DOM, indicate that a interaction is occurring between Hg, sulfide and DOM which is currently not accounted for in thermodynamic models. Two small model organic ligands, EDTA and thioglycolate, elicited the same response as DOM when added to solutions containing Hg and sulfide, indicating that functional groups, such as carboxylic acids, in the organic matter might be responsible for driving the interaction between Hg, sulfide and DOM. When added to solutions of Hg, sulfide and DOM, calcium, which should bind to and reduce the reactivity of carboxylic acid functional groups, did not change the measured D_{ow}'s. This questions the importance of functional groups, on the DOM, in controlling the interaction of DOM with Hg and sulfide. Using ultrafiltration, the interaction between Hg, DOM and sulfide was confirmed. The information gained from the ultrafiltration studies, also, suggests that DOM is only interacting with the neutral Hg-sulfide complexes. The data collected

from the ultrafiltration experiments using solutions of Suwannee River DOM, Hg and sulfide can be used to calculate the stability constant for the interaction of Hg, DOM and sulfide.

5.3.4. Calculation of conditional stability constants

Using the information gained about the mechanism behind the interaction of Hg-sulfide complexes and DOM, conditional stability constants can be calculated for the Hg-sulfide-DOM interaction using the ultrafiltration data. The interaction appears to be driven by the association of DOM with either $HOHgSH^0$ (Equation 4) or both $HOHgSH^0$ and $Hg(SH)_2^0$ (Equation 5), where Hg-$sulfide_N$ is the sum of $HOHgSH^0$ and $Hg(SH)_2^0$.

$$HOHgSH^0 + DOM = HOHgSHDOM \quad (4)$$

$$\text{Hg-sulfide}_N + DOM = \text{Hg-sulfide}_N DOM \quad (5)$$

$$K_N = [\text{Hg-sulfide}_N\text{-DOM}]/[\text{Hg-sulfide}_N][DOM] \quad (6)$$

$$K_{HOHgSH} = [HOHgSH^0DOM]/[HOHgSH][DOM] \quad (7)$$

The ultrafiltration data from the DOM gradient and sulfide gradient experiments were used to calculate the conditional stability constants (K_N and K_{HOHgSH}) using Equations 6 and 7 respectively. After ultrafiltration it can be assumed that all of the DOM associated Hg is in the form of Hg-sulfide-DOM. The Hg passing through and sorbing to the filter represents the total concentration of inorganic Hg-sulfide complexes although there is also a small fraction of the Hg associated with the DOM

that is also retained on the filter. Using the total inorganic Hg-sulfide concentration, and the complexation constants for the Hg-sulfide species (Table 1.1), the concentration of $HOHgSH^0$ and $Hg(HS)_2^0$ can be calculated using MINEQL+. The calculated distribution of these two Hg species along with the DOM concentration (mg C/L) and the DOM associated Hg concentration can be used to calculate the conditional stability constant. The conditional stability constants for six solutions were calculated (Table 5.1) and the average constant was 0.16 ± 0.04 L mg C^{-1} for K_N and 0.25 ± 0.21 L mg C^{-1} for K_{HOHgSH}. The six solutions covered a range in sulfide concentrations (2.9-269 μM) and also a DOM gradient (5.6-19.2 mg C/L). The similarity in the calculated K_N from the six solutions indicates that equation 4 represents the interaction of Hg-$sulfide_N$ with DOM fairly well, supporting the idea that only the neutral Hg sulfide complexes are reacting with the DOM. The larger variability in the calculated constant assuming $HOHgHS^0$ is the only Hg-sulfide reacting (K_{HOHgSH}), especially at high sulfide concentrations, provides evidence that the interaction involves both $HOHgHS^0$ and $Hg(HS)_2$ and not just $HOHgSH^0$. As noted above, some of the Hg sorbed to the filter is likely associated with DOM since it was found that up to 20% of the Hg sorbed to the filter when Hg-DOM complexes were the only complexes in solution. Therefore, the calculated stability constant is likely slightly underestimating the strength of the interaction of Hg-sulfide with DOM. It should also be noted that all of the solutions had a pH close to 6, so the effect of pH on this reaction can not be determined from these data and therefore the constants are conditional.

Table 5.1: Conditional stability constants for the interaction of Hg with Suwannee River DOM determined from ultrafiltration data. The conditional stability constants were determined for the interaction for $HOHgSH^0$ with DOM (K_{HOHgOH}) and also for the interaction of both $HOHgSH^0$ and $Hg(SH)_2^0$ with DOM (K_N).

DOM (mg C L^{-1})	**Sulfide (μM)**	**pH**	**Distribution of Hg-sulfide complexes**		**K_{HOHgSH} (L mg C^{-1})**	**K_N (L mg C^{-1})**
11.7	2.9	6.1	$HOHgSH^0$	88%	0.23	0.23
			$Hg(HS)_2^0$	2%		
			HgS_2H^-	9%		
11.7	23	6.1	$HOHgSH^0$	49%	0.16	0.13
			$Hg(HS)_2^0$	10%		
			HgS_2H^-	40%		
11.7	269	6.1	$HOHgSH^0$	8%	0.68	0.20
			$Hg(HS)_2^0$	19%		
			HgS_2H^-	73%		
5.9	7.8	6.3	$HOHgSH^0$	68%	0.15	0.14
			$Hg(HS)_2^0$	4%		
			HgS_2H^-	7%		
12.1	7.8	6.3	$HOHgSH^0$	68%	0.13	0.13
			$Hg(HS)_2^0$	4%		
			HgS_2H^-	7%		
19.2	7.8	6.3	$HOHgSH^0$	68%	0.13	0.12
			$Hg(HS)_2^0$	4%		
			HgS_2H^-	7%		
			Average		**0.25 (±0.21)**	**0.16 (±0.04)**

While Reaction 4 provides a good representation of the interaction between neutral Hg-sulfide and DOM this equation is not very useful in predicting the association of DOM with Hg-sulfide in the environment, since the concentration of the neutral Hg-sulfide species are not easily measured. In order to write this reaction in a more useful form, it needs to be assumed that $HOHgSH^0$ and $Hg(SH)_2^0$ are reacting with the DOM to the same extent. This is supported by the fact that the calculated K value does not change significantly over the range of sulfide concentrations. At the lowest sulfide concentration $HOHgSH^0$ dominates the neutral

species, while at the highest sulfide concentration $Hg(SH)_2^0$ becomes more important. If there was a difference in the reactivity of these species to DOM, the calculated constants would not be in agreement over the range of sulfide concentrations. Two separate reactions can therefore be written to describe the interaction of Hg-sulfide complexes with DOM both having the same stability constant (Reactions 8 and 9).

$$HOHgSH^0 + DOM \leftrightarrow (HOHgSH)DOM \qquad K = 0.16 \text{ L mg } C^{-1} \qquad (8)$$

$$Hg(SH)_2^0 + DOM \leftrightarrow (Hg(SH)_2)DOM \qquad K = 0.16 \text{ L mg } C^{-1} \qquad (9)$$

These reactions can be rewritten using the formation reactions of $HOHgSH^0$ and $Hg(SH)_2^0$ (Table 1.1) so that they are expressed in terms of Hg^{2+}.

$$Hg^{2+} + HS^- + DOM + H_2O \leftrightarrow HOHgSHDOM + H^+ \qquad (10)$$

$$K = 10^{25.7} \text{ L mg } C^{-1}$$

$$Hg^{2+} + 2HS^- + DOM \leftrightarrow Hg(SH)_2DOM \qquad (11)$$

$$K = 10^{36.7} \text{ L mg } C^{-1} \qquad (11)$$

These equations can be used in equilibrium models to predict the speciation of Hg under sulfidic conditions.

The similarity in the reactivity of both $HOHgSH^0$ and $Hg(HS)_2^0$ also provides more information on the mechanism controlling the interaction of Hg-sulfide with DOM. Hg is a soft sphere metal with highly polarizable d shell electrons, resulting in Hg having a higher affinity for sulfur ligands relative to oxygen containing ligands. Mercury preferably forms two-coordinate, linear complexes (Cotton et al. 1995). Therefore, in order for DOM to form a complex with Hg-sulfide species, it would

have to out compete one of the ligands in the Hg-sulfide complex. While thiol groups on the DOM potentially could displace the OH group in the HOHgHS complex, they will not likely displace the Hg-S bond. Since both $HOHgSH^0$ and $Hg(SH)_2^0$ are interacting with the DOM, this interaction can not be explained readily by the displacement of a hydroxyl group by the DOM. It is therefore, not likely that DOM is forming linear ligand-metal complex with the neutral Hg-sulfide species. Rather, the interaction appears to be best described as a partitioning of the neutral Hg-sulfide into the DOM, which could be driven by a hydrophobic interaction between the non-polar portions of the DOM and the neutral Hg-sulfide complexes. The lack of interaction observed between DOM and charged Hg-sulfide complexes provides further support for a partitioning interaction.

It was shown using D_{ow} extractions, that EDTA and thioglycolate reduced the formation of neutral Hg-sulfide. These results do not support the proposed mechanism of neutral Hg-sulfide interaction with nonpolar portions of DOM since both EDTA and thioglycolate are small charged organic molecules. This same discrepancy was observed in studies examining the influence of DOM in the precipitation and dissolution of $HgS_{(s)}$, suggesting that while eliciting the same response, small model organic ligands might not provide the best means of investigating the interaction of DOM with Hg and sulfide. Indeed, as these experiments were relatively limited in scope and did not examine the complexation over a range of sulfide concentrations, it cannot be assumed that the interaction is between both neutral complexes and the thioglycolate and EDTA. Indeed, in these experiments, HOHgSH dominated as the neutral complex (~60% of the total) with

$Hg(SH)_2$ as a small fraction (~10%). Thus, in these simple solutions and with small molecules, it may be reasonable to consider that only the HOHgSH is interacting via an exchange reaction with the –OH group (Reaction 12 and 13) where $RCOO^-$ represents EDTA or another carboxylic acid group.

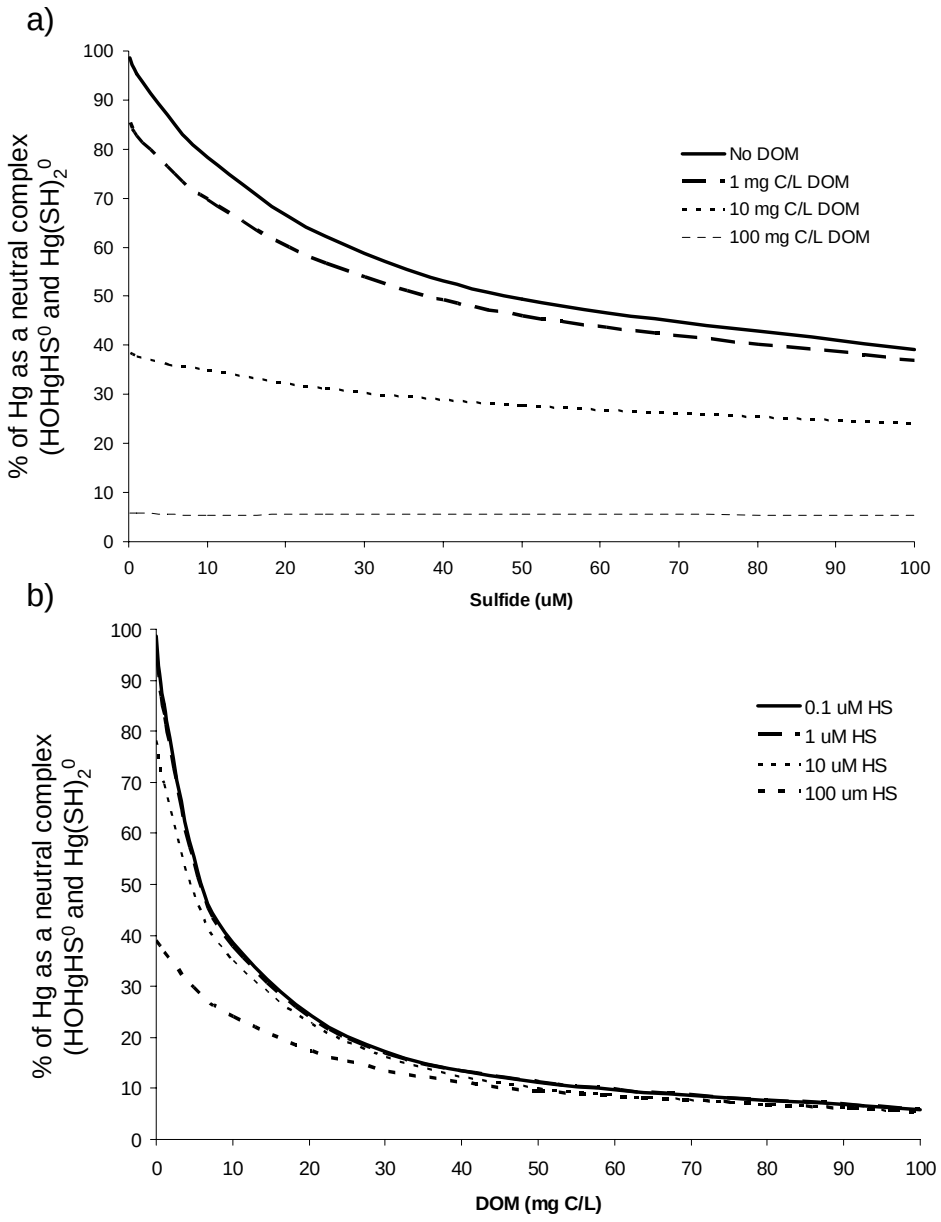
$$HOHgSH + HSCH_2COO^- = {}^-OOCCH_2SHgSH + H_2O \quad (12)$$

$$HOHgSH + RCOO^- = RCOOHgSH + OH^- \quad (13)$$

5.4. Application of stability constants to natural systems

The stability constants for the interactions of neutral Hg-sulfides with Suwannee River DOM were added to the MINEQL+ database and used to calculate Hg speciation across a range of environmentally relevant DOM and sulfide concentrations (Figures 5.8), at pH 6. Prior thermodynamic models have not included the interaction of DOM and Hg-sulfide observed here. Since measured stability constants for Hg-DOM complexes in the absence of sulfide are very low relative to the stability constants of Hg-sulfide complexes (Ravichandran 2004), prior models have shown little impact of DOM on Hg complexation in the presence of sulfide. In models that do not include a Hg-sulfide-DOM interaction, neutral Hg-sulfide species dominate the dissolved speciation of Hg at low μM sulfide

Figure 5.8: Modeled speciation of Hg, presented as the % of dissolved Hg complexes which are neutral, across a) sulfide and b) DOM gradient, using the stability constants for the interaction of Hg-sulfide with DOM determined in this study. In graph (a) the solid line (No DOM) is analogous to previously used thermodynamic models which did not include the interaction with DOM.



concentrations, while charged Hg-sulfide species dominate once sulfide concentrations reach roughly 100 μM (Benoit et al. 1999a; Benoit et al. 1999b). The inclusion of Hg-sulfide-DOM complexes in thermodynamic models significantly reduces the fractional abundance of free neutral Hg-sulfide complexes, in a concentration-dependant manner. Based on the constants calculated in this study, at DOM concentrations above 10 mg C/L, the abundance of neutral Hg-sulfide species does not change significantly over a range of sulfide concentrations. Both sulfide and DOM concentrations are important in controlling the abundance of neutral Hg-sulfide. It should be noted that modeled abundance of neutral Hg-sulfide in the presence of DOM is largely a function of the magnitude stability constants for the formation of Hg-sulfide-DOM species. The stability constants used in the model presented here are based on experiments using only one type of DOM. The stability constants for Hg-DOM in the absence of sulfide range several orders of magnitude, partly as a result of difference in the DOM characteristics used to calculate the constants (Ravichandran 2004). It is very likely that the stability constants for the interaction of DOM with Hg-sulfide using different DOM would also vary based on the DOM characteristics and this variability would impact the modeled speciation of Hg.

The calculated stability constants were also applied to model the porewater speciation of Hg in samples collected from the San Francisco Bay Delta in California and the Florida Everglades. The speciation of Hg was calculated for sites spanning a spatial gradient across the Florida Everglades (Figure 5.9) which contained a range of

Figure 5.9: Sites in the Florida Everglades in which the speciation of Hg was determined using the complexation constant for the interaction of DOM with the sulfide speciation

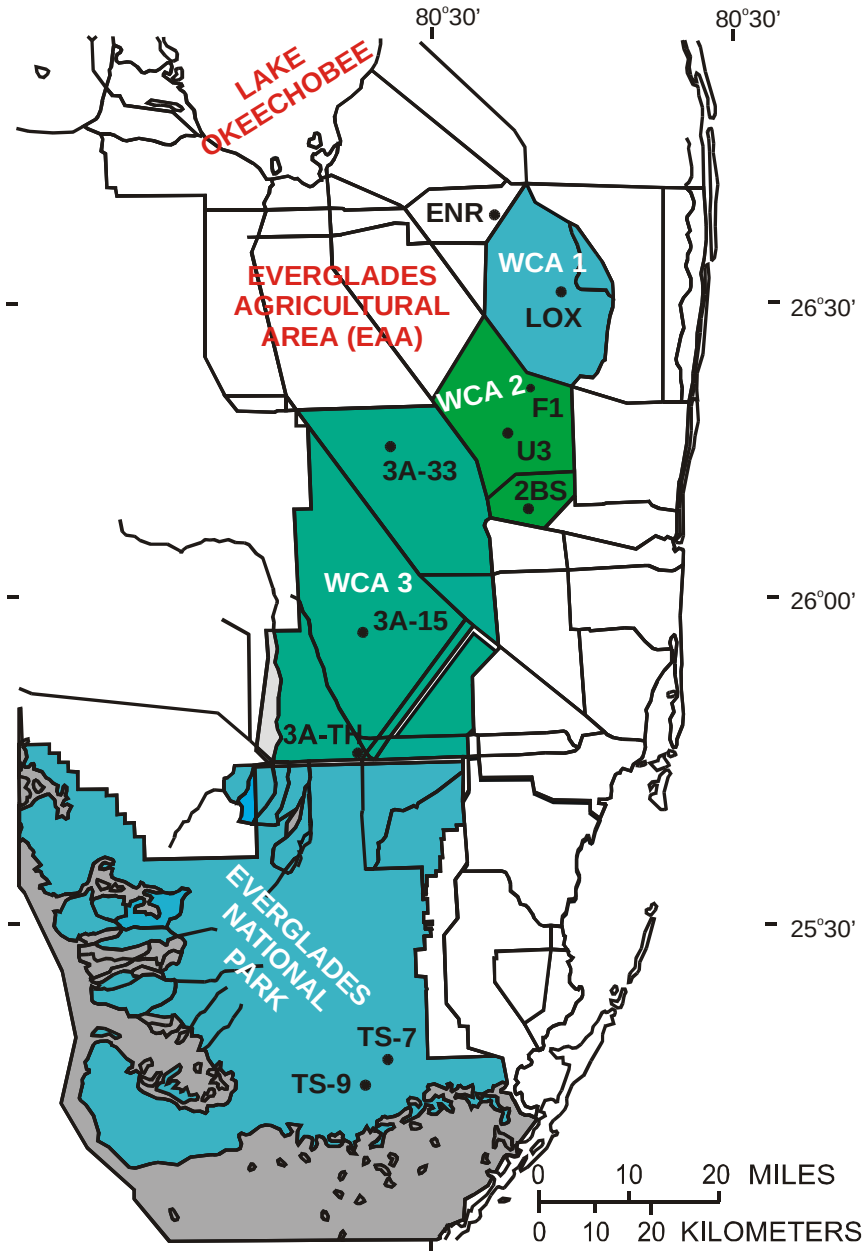
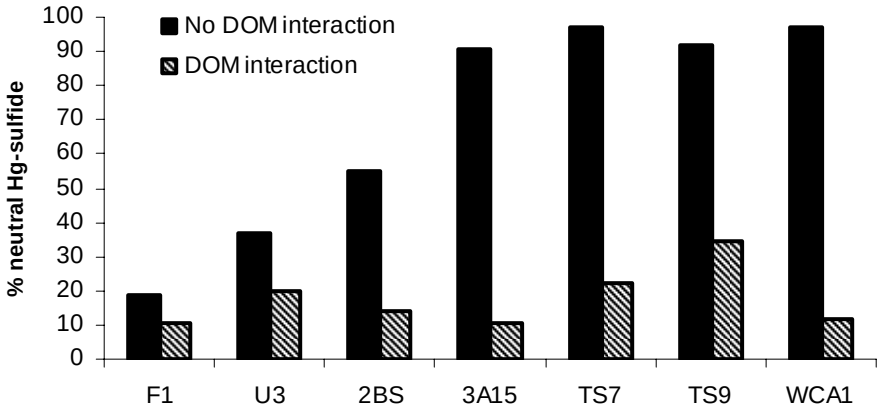
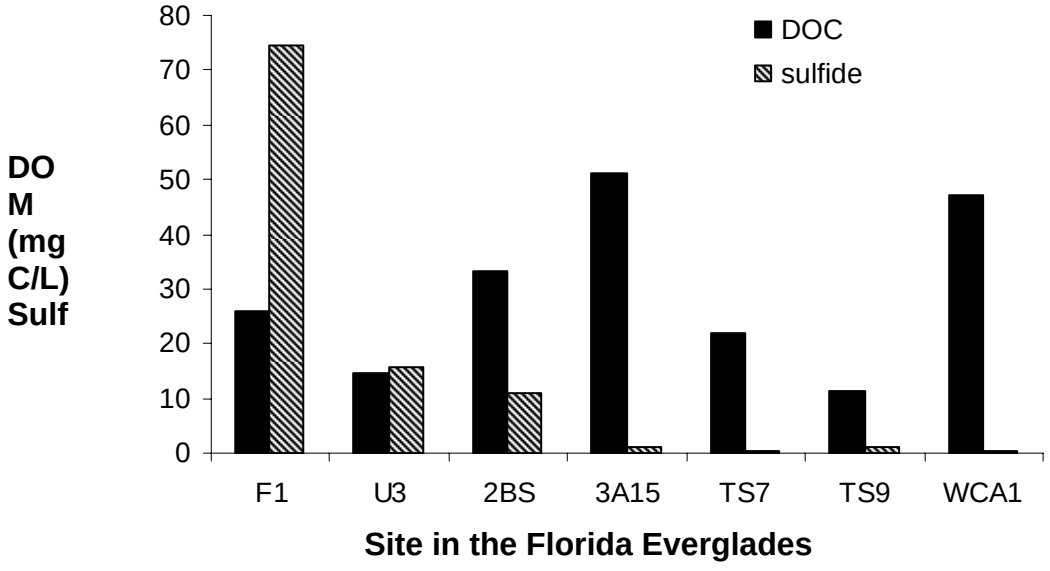


Figure 5.10: a) Calculated abundance of neutral Hg-sulfide complexes across sites in the Florida Everglades with and without the interaction DOM on the complexation included in the model. b) DOM and sulfide data used to calculate the speciation of Hg





sulfide and DOM concentrations (Figure 5.10b). Average dissolved phase Hg, sulfide and DOM concentrations from the top 4 cm of peat cores collected between 1995 and 1998 were used in the speciation calculations. In general from north to south in the Florida Everglades the sulfide and DOM concentration decreases. Inclusion of the interaction of DOM with Hg-sulfide complexes results in a decrease in the predicted abundance of neutral Hg-sulfide by 44-88% in the samples collected across a large special gradient in Florida Everglades (Figure 10a) with the concentration of DOM becoming more important than sulfide in driving the interaction (Figure 10b). The interaction of DOM with the Hg-sulfide complexation not only decreases the predicted abundance of neutral Hg-sulfide but also decrease the variability of these species across sites. Similar results were found for porewater samples collected from the San Francisco Bay Delta, in which the inclusion of DOM in the speciation calculations reduced the neutral Hg-sulfide complex abundance by 73-91%. The greatest reduction in neutral Hg-sulfide in both the Florida Everglades and the San Francisco Bay Delta was observed in regions with low micromolar sulfide concentrations as a result of the dominance of $HOHgSH^0$ under these conditions.

It is very likely that differences in the composition of DOM across and within ecosystems will impact the extent in which the Hg-sulfide-DOM complex forms. The stability constants used in the thermodynamic calculation were only determined using one DOM isolate, therefore the modeled data only provides an indication of how DOM could affect the complexation of Hg under sulfidic condition. At this point, the characteristics of the DOM which are important in the formation of the Hg-sulfide-DOM species are not clearly understood. If neutral Hg-sulfide complexes are

partitioning into DOM, the interaction of Hg-sulfide with DOM would be stronger when more hydrophobic DOM is present. The data presented here suggest that carboxyl and thiol functional groups could also be important in the interaction of between Hg, sulfide and DOM and therefore, the abundance of these functional groups on DOM could also be significant in controlling the complexation of Hg under sulfidic conditions. In order to more accurately predict the complexation of Hg in natural samples a better understanding of the interaction of DOM with Hg-sulfide complexes in needed.

5.5. Implication for Hg methylation

The abundance of neutral Hg-sulfide complexes has been suggested to be a controlling factor in the bacterial conversion of Hg to MeHg by sulfate reducing bacteria. The uptake of Hg by Hg-methylating sulfate-reducing bacteria appears to depend on the concentration of dissolved neutral Hg-sulfide species, and diffusion has been postulated as the uptake mechanism (Benoit et al. 2001b). Further, Hg methylation rates *in situ* have been correlated with the calculated concentrations of neutral Hg-sulfide species in sediment and soil porewaters (Benoit et al. 1999b). Benoit et al. (2003) estimated the diffusive uptake rate of neutral Hg-sulfide species into bacterial cells based on measured D_{ow} values. These calculations suggested that the rate of neutral Hg-sulfide species entering the cell was several orders of magnitude greater than measured Hg methylation rates in bacterial cultures (Benoit et al. 2001b). However, these calculations were based on the predicted thermodynamic speciation of Hg that did not include the interaction of Hg-sulfide with DOM.

Including DOM in the speciation calculations results in a reduction in the predicted abundance of neutral Hg sulfide species, which in turn results in a decreased diffusion rate of Hg into the bacterial cells (since diffusion is concentration dependent). While the predicted abundance of neutral Hg-sulfide is reduced as a result of including the interaction with DOM, the concentration would still be in excess of the concentration needed to provide sufficient Hg to the bacteria to account for the measured Hg methylation rates in pure cultures of sulfate reducing bacteria (Benoit et al. 2001b).

Applying the stability constants for the interaction of Hg-sulfide with DOM to a range of DOM and sulfide concentrations indicated that DOM concentrations are important in controlling the speciation of dissolved Hg under typical environmental conditions. Differences in sulfide concentrations have been used to explain some of the variability in MeHg production found within and between ecosystems. The results from this study suggests that the DOM concentration in ecosystems might be as important, if not more important, than sulfide in controlling the speciation of Hg under some conditions. The mechanism of the DOM- HgS interaction needs further study before the influence of DOM on Hg methylation can be adequately modeled. These mechanisms can be studied by examining the interaction of Hg-sulfide with DOM isolates with a range of characteristics, including the concentration of key functional groups and the degree of hydrophobicity, as suggested in this chapter. The impact of DOM on Hg uptake and methylation by microorganisms should also be examined directly.

Chapter 6: Chapter 6: Conclusions

6.1. Overview

The interaction of Hg with organic matter is extremely significant in controlling the dissolved phase speciation and solid phase partitioning of Hg. Previously, the interaction of Hg with DOM has been shown as an important factor in the mobility and reactivity of Hg throughout oxic portions of aquatic systems (Ravichandran 2004) but its influence on Hg dynamics under sulfidic conditions has largely been ignored. Also, the interplay between organic matter and iron solids in controlling the particle association of Hg had not been given much attention. As a result of the importance of Hg complexation in the uptake and subsequent methylation of Hg my sulfate reducing bacteria (SRB), understanding the dissolved phase speciation of Hg under sulfidic conditions is a crucial step in elucidating the processes controlling the production of MeHg across ecosystems (Benoit et al. 2003). The distribution of Hg between the solid and dissolved phase also has significant implications in the methylation of Hg by SRB since it is thought that bacteria take up Hg from the dissolved phase, which accounts for only a small fraction of Hg in the sediment.

6.2. Research Hypotheses

My dissertation research addresses four hypotheses:

1) In the presence of solid Fe sulfide phases, Hg and MeHg will strongly sorb to the solid phase because Hg and MeHg form strong complexes with reduced sulfide ligands
2) The presence of dissolved organic matter in natural systems will reduce the sorption of Hg and MeHg to iron oxides in a concentration-dependant manner, because Hg and MeHg complexation with DOM is stronger than with iron oxides
3) The presence of dissolved organic matter in natural systems will not affect the sorption of Hg and MeHg to solid iron sulfides, because Hg and MeHg complexation with DOM is much weaker that with iron sulfide
4) The presence of dissolved organic matter in natural systems will not affect the speciation of dissolved mercury under sulfidic conditions, because the complexation of Hg with DOM is much weaker than with dissolved sulfides

6.3. Influence of iron, sulfur and organic matter on Hg and MeHg solid phase partitioning

Using laboratory experiment to examine the association of Hg and MeHg with hydrous ferric oxide (HFO) and amorphous iron sulfide ($FeS_{(s)}$) in the presence and absence of DOM, the first three research hypotheses were shown to be correct. The combined affect of iron, sulfur and organic matter needs to be considered in order to understand the association of Hg and MeHg with particles. This study (Chapter 2), along with previous research (Dzombak and Morel 1990; Tiffreau et al. 1993; Collins et al. 1999; Backstrom et al. 2003), showns that Hg has a high affinity of

HFO in the absence of organic matter, but organic matter changes this interaction. This is a result of the stronger interaction of Hg with DOM relative to the surface sites on the Fe oxide. Hg preferably complexes with DOM over HFO surface sites and, therefore, the association of Hg with Fe oxide in the presence of DOM is controlled by the interaction of DOM with the Fe oxide. The absorption of MeHg to HFO is weaker than that of Hg, and DOM did not impact this interaction in the same manner as was observed with Hg.

Both Hg and MeHg formed strong complexes with amorphous $FeS_{(s)}$ as a result of the strong association of Hg and MeHg to reduced sulfur surface sites on the $FeS_{(s)}$. While organic matter influenced the interaction of Hg and MeHg with HFO, this was not observed with amorphous $FeS_{(s)}$. The influence of DOM on the sorption of Hg and MeHg to HFO was a result of the stronger affinity of Hg and MeHg to DOM relative to the surface functional groups on the HFO. The importance of DOM in this interaction was enhanced as a result of the complexation of DOM to the surface of the HFO. While it is not know if DOM associated with $FeS_{(s)}$, this interaction should not affect the sorption of Hg to $FeS_{(s)}$. Hg form strong complexes with dissolved phase sulfide and reduced sulfur surface sites on the $FeS_{(s)}$ and, therefore, these interactions will dominate over the interaction of Hg with organic matter. The complexation constant of MeHg with $FeS_{(s)}$, calculated in this study, is similar to previously measured complexation constants for MeHg to organic matter (Amirbahman et al. 2002; Qian et al. 2002; Karlsson and Skyllberg 2003). As a result, the solid phase association of MeHg under sulfidic conditions might be a function of the interaction

of MeHg with both $FeS_{(s)}$ and organic matter, and the dominant interaction will depend on the relative concentration of each in the sediment.

In natural aquatic systems, Fe solids and organic matter do not exist as separate entities (Mayer 1999), and from the research presented in Chapter 2, it is evident that to understand the particle association of Hg and MeHg, the synergistic effect of Fe solids and organic matter needs to be considered. Fe solids, which undergo dynamic redox changes in aquatic systems, are important in the particle association of both Hg and MeHg. Since redox interfaces have been shown as important sites for Hg methylation (Benoit et al. 2003), changes of the particle association of Hg at redox boundries, as a result in changes in the Fe particle composition, could impact Hg methylation.

6.4. Dissolved phase speciation of Hg under sulfidic conditions

The fourth research hypothesis stated that the presence of dissolved organic matter in natural systems will not affect the speciation of dissolved mercury under sulfidic conditions, because the complexation of Hg with DOM is much weaker than with dissolved sulfides. This hypothesis was also shown to be incorrect. Previously, it was thought that organic matter did not impact the speciation of Hg under sulfidic conditions as a result of the much stronger interaction of Hg with sulfide relative to DOM. Using modified octanol-water partitioning (D_{ow}) techniques (Chapter 3), the concentration of neutral Hg-sulfide was shown to be much lower than predicted by thermodynamic models. D_{ow} extractions and centrifugal ultrafiltration of laboratory prepared solutions containing Hg, sulfide and DOM demonstrated that the lower

concentration of neutral Hg-sulfide found in natural samples relative to thermodynamic predictions was a result of the influence of DOM on the speciation of Hg under sulfidic conditions (Chapter 4).

In order to understand the mechanism controlling the interaction between Hg, sulfide and DOM, additional laboratory experiments were conducted using model organic ligands and DOM isolates, both in the presence and absence of sulfide. From these experiments it was demonstrated that only neutral Hg-sulfide complexes are interacting with DOM, and it is proposed that this interaction is a result of the partitioning of the neutral Hg-sulfide complexes into hydrophobic portions of the DOM. Stability constants were determine to describe the interaction of DOM with Hg-sulfide and these constants were then used in thermodynamic models to examine the influence of DOM on Hg complexation under environmentally relevant conditions. The models suggest that the abundance of neutral Hg-sulfide is influenced by both the DOM concentration and the sulfide concentration, with their relative importance being a function of the relative concentration of each ligand in solution. The thermodynamic model was also used to predict the speciation of Hg at two different locations, the Florida Everglades and the San Francisco Bay Delta, demonstrating the importance of DOM on the speciation of Hg over a range of sulfide and DOM concentrations. All of the data indicates that the abundance of neutral Hg-sulfide is less than previously thought as a result of the interaction with DOM (Chapter 5).

In addition, while the current notion that Hg methylation is hindered at high sulfide concentrations may be correct within an ecosystem of relatively constant

DOM, comparisons of the relative magnitude of methylation across ecosystems may not be simply made based on sulfide concentration alone. For example, the results presented here may provide a partial explanation for the observation that methylation rates in estuarine and salt marsh environments, where sulfide concentrations are typically higher, are not substantially different than those in lower sulfide freshwater locations. In freshwater ecosystems, DOM levels are often higher than those of coastal regions and thus, the interaction described above, where higher DOM levels leads to a decrease in the neutral sulfide concentration, will tend to counteract the lowering of the neutral sulfide concentration that occurs with the higher sulfide levels of coastal regions.

6.5. Implications for Hg methylation

Diffusive uptake of neutral Hg-sulfide has been shown to be important in the methylation of Hg by sulfate reducing bacteria (Benoit et al. 1999b; Benoit et al. 2001c). The concentration of the Hg complexes, available for diffusive uptake, is a function of the total concentration of Hg in the porewater and also the speciation of that Hg (Benoit et al. 2003). The particle affinity of Hg will control the concentration of Hg in the dissolved phase, and from the research presented in Chapter 2, it is obvious that Fe solids are important in the association of Hg with solids. Active zones of Hg methylation have been shown to occur at the redox interfaces in aquatic systems. High methylation rates in these regions are likely a result of the increased activity of sulfate reducing bacteria coupled with the appearance of sulfide in the porewater (Benoit et al. 2003). In these regions the composition of particles is also

changing, as a result in the shift from Fe oxide particles to Fe sulfide particles. Since Hg interacts with $FeS_{(s)}$ directly and Fe oxides indirectly through the association with organic matter, the solid phase partitioning of Hg is likely also undergoing changes at the redox interface which could impact the dissolved phase concentration and bioavailablity of Hg.

Previous studies have not considered an interaction of dissolved Hg-sulfide complexes with DOM and, therefore, the abundance of neutral Hg-sulfide has been overestimated. The diffusive uptake rates of neutral Hg-sulfide, by sulfate reducing bacteria, have previously been calculated using the K_{ow} of neutral Hg-sulfide complexes. These rates were shown to be several orders of magnitude higher than measured Hg methylation rates in the same systems. It was speculated that the discrepancy between the estimated uptake rate of Hg and the measured methylation rate was a result of the sequestration of Hg inside the cell, making it unavailable for methylation (Benoit et al. 2003). The concentration of neutral Hg-sulfide outside the cell is one of the variables in the diffusive uptake rate calculations. The interaction of Hg-sulfide with DOM result in a reduction in the predicted concentration of neutral Hg-sulfide, which, in turn, results in a decrease in the diffusive uptake rate of Hg into the cells of SRB. As mentioned before, the calculated diffusive uptake rate of Hg into the cell is several orders of magnitude larger than measured methylation rates. Including the interaction of Hg-sulfide with DOM decreases the abundance of neutral Hg-sulfide by, at most, 90%, and therefore, the diffusion of Hg into the cell should still provide sufficient Hg for the observed methylation rates.

Variability in MeHg production across and within ecosystems is a function of the bacteria activity and community composition, since MeHg production is controlled by sulfate reducing bacteria, and the chemical speciation of Hg (Benoit et al. 2003). Since DOM has been shown to strongly influence the speciation of Hg under sulfidic conditions, differences in the concentration and composition of DOM has the potential to impact the production of MeHg. The direct affect of DOM on Hg methylation has not been examined and, therefore, it is not known how the influence of DOM on the speciation of Hg will impact this process. Also, the interaction of DOM in the Hg-sulfide speciation was only examined using two different DOM isolates, so it is not known how the characteristics of DOM will affect this interaction.

6.6. Future work

While it is obvious that DOM influences the speciation of Hg under sulfidic conditions, more research is needed to determine how different DOM isolates influence Hg speciation. As mentioned above, only two DOM isolates, one from the Florida Everglades and the other from Suwannee River, were used to investigate the interaction of DOM on the speciation of Hg. We proposed that this interaction is a result of neutral Hg-sulfide partitioning into hydrophobic portions of DOM. In order to examine this proposed interaction farther, the interaction of Hg-sulfide complexes with various DOM needs to be examined. Using ultrafiltration, an initial attempt was made to examine the influence of DOM characteristics on the DOM interaction with Hg-sulfide complexes. Ultrafiltration was conducted on solutions prepared with eight

different DOM isolates in the presence and absence of sulfide. While it appears that the interaction of the DOM with Hg-sulfide complexes varied with the different DOM, the data was difficult to interpret as a result of significant adsorption of the Hg-DOM complexes to the ultrafilters. This sorption was much greater than observed with other experiments using ultrafiltrations and, therefore, for this experiment to provide useful results, it needs to be repeated.

Determining the stability constants for the interaction of different DOM isolates with Hg-sulfide complexes will enhance the ability of thermodynamic models to predict the speciation of Hg under sulfidic conditions. These models would also be improved if the influence of pH on the interaction of Hg-sulfide with DOM is examined. As mentioned in Chapter 5, the stability constant for the interaction of Suwannee River DOM with neutral Hg-sulfide complexes was only examined at pH 6. Since the overall charge of DOM will change with pH, the influence of DOM on Hg-sulfide complexation needs to be examined over a range of environmentally relevant pH's. This type of experiment could be conducted using the ultrafiltration approach used to determine the stability constants in Chapter 5.

The interaction of DOM on Hg methylation has never been directly examined, since previously DOM was not thought to be an important player in this process. As a first cut at examining the influence of DOM on Hg methylation, controlled experiments, using sulfate reducing bacteria known to methylation Hg, should be conducted using different DOM concentrations. Methylation experiments, using bacterial cultures, should also be done using a suite of well characterized DOM to examine how different DOM characteristics affect methylation rates.

Currently, it is common to measure the porewater concentration of dissolved Hg and sulfide in studies designed to examine the production of MeHg, but DOM is not always measured. As a result of the importance of DOM on the complexation of Hg under sulfidic conditions, DOM also needs to be measured in these systems so that it can be included in thermodynamic speciation models. This will hopefully provide a better understanding of the influence of DOM on Hg methylation in natural systems.

In order to further examine how redox changes affect the partitioning of Hg between the solid and dissolved phase, controlled laboratory experiments could be used to investigate this partitioning. Sediments with known Fe content could be amended with organic matter to create a series of solid phases with a constant Fe content that spanned a range of organic contents. The distribution of Hg, between the solid and dissolved phases, in these sediments under oxic conditions could initially be examined. The sediment could then be subjected to reducing conditions and changes in the dissolved phase Hg concentration could be examined over time as the dominant Fe solid shifts from Fe oxide to Fe sulfide. This would provide a controlled set of conditions to examine how the dissolved phase concentration of Hg changes at the redox interface in the sediment.

Overall, more experiments need to be done examining the influence of organic matter on the biogeochemistry of Hg and MeHg, especially under sulfidic conditions. Laboratory studies provide a first means to examine this interaction, but it should be noted that isolated organic matter used in these experiments is likely very different

from the organic matter in natural samples. Therefore, it is also important to apply what is learned in the laboratory to natural systems.

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